“Studies on the potential association of GRIN2B and DRD4 variants with cognitive scores of Wilson disease patients, and updating disease data for the Indian Genetic Disease Database”

Project report submitted to Sister Nivedita University in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE
IN
MICROBIOLOGY

Submitted By
PRAKRITI JANA
Registration ID: 230010253750
Enrollment ID: 2322211001025

Advisor
Dr. Kunal Ray
Director, ATGC Diagnostics Pvt. Ltd.
Kolkata

School of Health Science and Translational Research
Sister Nivedita University
Kolkata

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Prakriti Jana**, a student of the Department of Microbiology, Sister Nivedita University, has successfully completed her long-term training **at ATGC Diagnostics Pvt. Ltd., West Bengal, India**, in the domain of Molecular Biology, from **4th January 2025 to 16th May 2025.**

During this period, she worked on a research project titled **"Studies on potential association of GRIN2B and DRD4 variants with cognitive scores of Wilson disease patients, and updating disease data for Indian Genetic Disease Database"** under the supervision of **Dr. Kunal Ray**, Principal Investigator of the project.

Ms. Jana was trained in key molecular biology techniques such as genomic DNA isolation, PCR, RFLP, gel electrophoresis, genotyping, and statistical data analysis for SNPs. She also assisted in updating data for the Indian Genetic Disease Database. She exhibited commendable enthusiasm, responsibility, and professionalism throughout the training period.

We appreciate her contribution and wish her all the best for her future endeavours.

01/06/2025

Dr. Kunal Ray

(Director)

Dr. Debdutta Bandyopadhyay

(Chief Training Officer)

21/2A, Gorachand Road, Kolkata - 700014 ATGC DIAGNOSTICS (P) LTD. Call : 7044776862

Mob/WhatsApp: 7044776862 Email Id: info@atgcdiagnosticsindia.com Website: https://atgcdiagnosticsindia.com/

REGD. OFFICE: 21/2A, Gorachand Road, Park Circus, Kolkata-700014, West Bengal, India

CIN: U85100WB2015PTC208619

DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "**Studies on the potential association of GRIN2B and DRD4 variants with cognitive scores of Wilson disease patients, and updating disease data for the Indian Genetic Disease Database**" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Microbiology is an authentic record of my own work carried out during the period from 4th January 2025 to 16th May 2025 under the supervision of Dr. Kunal Ray, Department of Research and Development, ATGC Diagnostics Pvt. Ltd. Kolkata.

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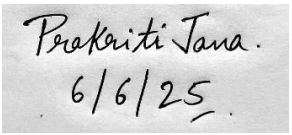
I further declare that no portion of the dissertation or its data will be published without the Institute's or supervisor's permission. I have not previously applied for any other degree or award using the topics and findings described in my dissertations.



(Signature of the students with Date)

Name of the Student: PRAKRITI JANA

Registration no.: 230010253750

Department of Microbiology

SISTER NIVEDITA UNIVERSITY
সিস্টার নিবেদিতা বিশ্ববিদ্যালয়

DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

TO WHOM IT MAY CONCERN

This is to certify that the project report titled "**Studies on the potential association of GRIN2B and DRD4 variants with cognitive scores of Wilson disease patients, and updating disease data for the Indian Genetic Disease Database.**", submitted by Ms. PRAKRITI JANA (Registration No: 230010253750, Enrolment No: 2322211001025) to the Department of Microbiology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by her for the partial fulfillment of the requirements for the Master of Science degree.

Date: 06.06.2025

Place: Newtown, Kolkata

Signature of Head of the Department

ACKNOWLEDGEMENT

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ABREVIATIONS

SL. NO.	ABBREVIATION	FULL FORM
1	WD	Wilson Disease
2	ATP7B	ATPase Copper Transporting Beta
3	SNP	Single Nucleotide Polymorphism
4	GRIN2B	Glutamate Receptor, ionotropic, N-methyl-D-aspartate 2B
5	DRD4	Dopamine Receptor D4
6	PCR	Polymerase Chain Reaction
7	RFLP	Restriction Fragment Length Polymorphism
8	IGDD	Indian Genetic Disease Database

ABSTRACT

My studies have two parts, as mentioned in the title of the thesis. Hence, the abstract is presented below as appropriate.

(1) Wilson Disease (WD) is a rare, autosomal recessive disorder caused by mutations in the *ATP7B* gene, resulting in impaired copper transport and toxic accumulation of the metal in the liver, brain, and other organs. While hepatic and neuropsychiatric manifestations are well-characterized, the cognitive aspects of WD remain underexplored. Not all patients with similar *ATP7B* mutations exhibit the same cognitive outcomes, suggesting a possible role of modifier genes in influencing cognitive phenotypes. This study investigates the potential association between cognitive performance and genetic polymorphisms in two neuronal genes—*GRIN2B* and *DRD4*—in clinically diagnosed WD patients. *GRIN2B* encodes the NR2B subunit of the NMDA receptor, which is critical for synaptic plasticity and cognitive function. *DRD4* encodes the dopamine D4 receptor, which plays a role in attention, executive function, and behavior. Both genes have been implicated in neuropsychiatric and cognitive disorders.

DNA was extracted from blood samples of WD patients, followed by PCR amplification of selected SNP regions in GRIN2B and DRD4 variants. Restriction digestion was carried out using restriction enzyme AciI for GRIN2B and EcoRI for DRD4, followed by gel electrophoresis to determine the genotypes. Cognitive scores were assessed using standardized neuropsychological methods. Statistical analyses were performed to evaluate the relationship between SNP genotypes and cognitive outcomes. This study highlights the importance of considering genetic modifiers in understanding the neurocognitive variability in Wilson Disease, which may contribute to more personalized approaches in prognosis and therapeutic strategies.

(2) Indians, an endogamous population with a high incidence of recessive disorders, make up one-sixth of the world's population. However, the diseases that affect the Indian population frequently lack thorough documentation. To alleviate this challenge, the Indian Genetic Disease Database (IGDD) was released (http://www.igdd.iicb.res.in), an integrated and carefully curated repository of mutation data on common and rare genetic diseases affecting the Indian population; the database currently contains information on 498 diseases.

We have reviewed and compiled information from multiple publications and successfully updated the Indian Genetic Disease Database (IGDD) with curated disease data for 25 monogenic diseases and gene information for 8 monogenic diseases. This process involved extracting relevant data from literatures, and organizing the findings according to the IGDD submission format.

KEYWORDS

Wilson Disease; Polymorphism; Cognition; GRIN2B; DRD4; SNP Genotyping; Genetic Modifiers; Neurogenetics; Genotype–Phenotype Correlation, IGDD, Monogenic diseases; Indian population

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1. INTRODUCTION

Wilson Disease (WD) is a rare genetic disorder caused by alterations in the ATP7B gene, which disrupt normal copper metabolism and lead to its harmful buildup in the liver and other organs. It follows an autosomal recessive inheritance pattern. Clinical manifestations of WD range widely, encompassing hepatic, neurological, and psychiatric symptoms. While hepatic dysfunction often dominates the early clinical picture, neuropsychiatric features, particularly cognitive deficits, are increasingly being recognized as integral to the disease spectrum. However, the extent and nature of cognitive impairment in WD remains underexplored, with studies indicating variable involvement in domains such as memory, attention, fluency, and processing speed (Mukherjee et al., 2014). Notably, even among patients sharing similar *ATP7B* mutations, phenotypic variability in cognitive presentation is frequently observed, suggesting the involvement of modifier genes that may influence disease expression. Recent literature has focused on candidate genes involved in neurotransmission pathways, particularly those regulating dopaminergic and glutamatergic signalling, which play central roles in cognitive processing.

The dopamine receptor D4 (DRD4) gene, which encodes a G-protein-coupled receptor predominantly expressed in the prefrontal cortex, has been associated with attention, decision-making, and executive function. Studies have shown that both sequence variants and epigenetic modifications in DRD4 influence cognitive traits in various neuropsychiatric conditions, including ADHD and bipolar disorder (Dadds et al., 2016; Zhao et al., 2015). For example, Barral et al. (2017) identified a promoter region variant, rs3756450, associated with enhanced perceptual speed, emphasizing the role of dopaminergic pathways in cognitive modulation.

The GRIN2B gene encodes the NR2B subunit of the NMDA receptor, a key component in glutamate-mediated excitatory neurotransmission. NMDA receptors play a vital role in regulating synaptic plasticity and are essential for cognitive functions such as learning and memory. Variants in GRIN2B have been implicated in attentional control, decision-making strategies, and phasic alertness, as shown in studies by Schulz et al., (2012) and Ness et al. (2011). These findings underscore the gene's influence on cognitive function in both healthy and clinical populations.

Given this context, the present study investigates the potential association between cognitive scores and single nucleotide (SNP) polymorphisms in GRIN2B and DRD4 among Wilson disease patients. Understanding how these genes may modify cognitive outcomes could offer valuable insights into the neurogenetic mechanisms underlying phenotypic variability in WD and pave the way for more individualized management approaches.

Initially, it was challenging to access reliable data for evaluating the prevalence of specific genetic disorders in India, as well as understanding locus and mutation-level diversity and identifying recurrent mutations, and the extent of molecular studies done in relation to disease burden. It is imperative to establish a depository of mutational data rounded by specific clinical and other applicable information of cases from different regions of India to effectively address the situation. Indian Genetic Disease Database (IGDD) is a forum to record case-specific mutation diapasons of inheritable conditions in the Indian population that will help to develop individual tests and assays to detect mutations, diagnose inheritable conditions, and identify carriers. It is developed and maintained at Indian Institute of Chemical Biology (IICB), a unit of the Council of Scientific and Industrial Research. The first interpretation of the database was published online (Pradhan et al., 2011). Nonstop updates of present conditions and addition of new conditions are being done to make a streamlined better interpretation of IGDD.

1.1 OBJECTIVE

- To evaluate the association between cognitive scores and single nucleotide polymorphisms in the GRIN2B and DRD4 variants among Wilson disease patients.
- To genotype the selected SNPs using PCR–RFLP and classify patients based on their allelic variants to explore genotype–phenotype correlations.
- To identify the potential role of GRIN2B and DRD4 variants as cognitive modifier genes, aiming to understand their contribution to cognitive variability in Wilson disease.
- To update the Indian Genetic Disease Database with curated data from the Indian patient cohort, including genetic and clinical profiles.

2. REVIEW OF LITERATURE

Wilson disease (WD), an autosomal recessive disorder, results from mutations in the ATP7B gene, leading to impaired copper metabolism and accumulation in vital organs such as the liver and brain. Neurological manifestations in WD are often highly variable even among patients with similar ATP7B mutations, suggesting the influence of modifier genes on phenotypic expression.

Among potential modifier genes, GRIN2B, which encodes the NR2B subunit of the NMDA receptor, has emerged as a promising candidate due to its critical role in glutamatergic neurotransmission, learning, memory, and cognition. Schulz et al. (2012) demonstrated that GRIN2B polymorphisms, particularly rs1806201, significantly modulate tonic alertness without affecting other attention networks. Subjects carrying the C allele displayed faster reaction times, possibly due to more effective glutamatergic transmission and subsequent noradrenergic activation, which enhances cortical arousal and alerting mechanisms.

Similarly, Ness et al. (2011) investigated GRIN2B variants in the context of decision-making using the Iowa Gambling Task (IGT) and found a sex-dependent association of rs1806201 and rs1806191 SNPs with win-stay/lose-shift strategies. These behavioural correlates highlight GRIN2B's influence on adaptive cognitive responses and reward-related behaviour in healthy individuals. Such findings support the hypothesis that GRIN2B polymorphisms could modulate cognitive symptoms in neurogenetic conditions like Wilson disease.

On the other hand, DRD4, a dopamine receptor gene predominantly expressed in the frontal cortex and limbic regions, plays a significant role in modulating executive function, working memory, and attention. In a study by Zhao et al. (2015), the rs3758653 SNP in the DRD4 promoter showed significant associations with cognitive performance and was also implicated in psychiatric conditions like bipolar disorder. Patients carrying this variant exhibited impaired scores in executive functioning tests such as the Wisconsin Card Sorting Test (WCST), indicating the involvement of DRD4 variant in cognitive control pathways.

Additionally, Barral et al. (2017) reported that DRD4 polymorphisms are significantly associated with slower processing speed and executive dysfunction in cognitively healthy individuals. Their meta-analysis also confirmed that the cognitive impact of DRD4 variants is domain-specific and modulated by receptor binding efficiency and expression regulation.

Given that both GRIN2B and DRD4 varinats influence neural circuits underpinning cognition, they are strong candidates for modulating the cognitive phenotype in Wilson disease. These genes likely act through their respective glutamatergic and dopaminergic pathways, interacting with the copper accumulation-induced neuropathology in WD.

In conclusion, previous literature substantiates the role of GRIN2B and DRD4 variants' polymorphisms in regulating core cognitive domains such as attention, executive function, and decision-making. Their investigation of potential modifier genes in Wilson disease may provide new insights into genotype-phenotype correlations and cognitive outcomes in affected individuals.

Indian Genetic Disease Database is focused on the data collection, curation, and arrangement of uncommon monogenic disorders in the Indian population based on the disease of interest, the causal gene, the nature of the mutations, and the geographic location of the patients or carriers, among other factors as said by Pradhan et al. (2011). By combining vast amounts of functional and evolutionary data and creating usable tools, the related research community will get access to a valuable resource and flexible platform.

3. MATERIALS AND METHODS

3.1 Study Design and Sample Collection

This study was designed to investigate the potential association of GRIN2B and DRD4 variants with cognitive scores of Wilson Disease (WD) in Indian patients. For GRIN2B variant, DNA samples of 45 clinically diagnosed Indian WD patients were analyzed. For DRD4 variant, a total of 50 DNA samples were analyzed.

The WD patient samples were obtained from the Department of Neurology at Bangur Institute of Neurosciences (BIN), IPGMER & SSKM Hospital, Kolkata, West Bengal, India, following ethical guidelines and with proper informed consent from the patients and their families.

3.2 DNA Extraction

Genomic DNA was extracted by the scholars in University of Calcutta, Department of Genetics, from whole blood samples using standard phenol-chloroform extraction method. The purity and concentration of DNA were assessed using a NanoDrop spectrophotometer by measuring absorbance at 260/280 nm. Each samples had a concentration of 35-40ng/ul and were stored at -20°C.

3.3 GRIN2B variant

3.3.1 Primer Designing

Primers were designed with the help of NCBI primer blast tool to amplify a **466 bp** region containing the polymorphic site within the GRIN2B variant's genomic sequence.

- Forward Primer: 5'-TGACCTCAGCTCACCACATG-3'
- Reverse Primer: 5'-GGCGGGTTGTTGTAGGATTT-3'

Fig - 01 [GRIN2B PRIMERS] (Source – SnapGene tool)

The melting (Tm) of the primers were calculated by the standard 2 (A+T) + 4 (G+C) formula. The Tm of the GRIN2B primers were calculated to be 61°C.

3.3.2 PCR Amplification

A specific segment of the GRIN2B variant was amplified using PCR.

Three PCR reactions were set up in a gradient manner with annealing temperatures below the Tm = 61°C. The temperatures considered were 57°C, 58°C and 59°C.

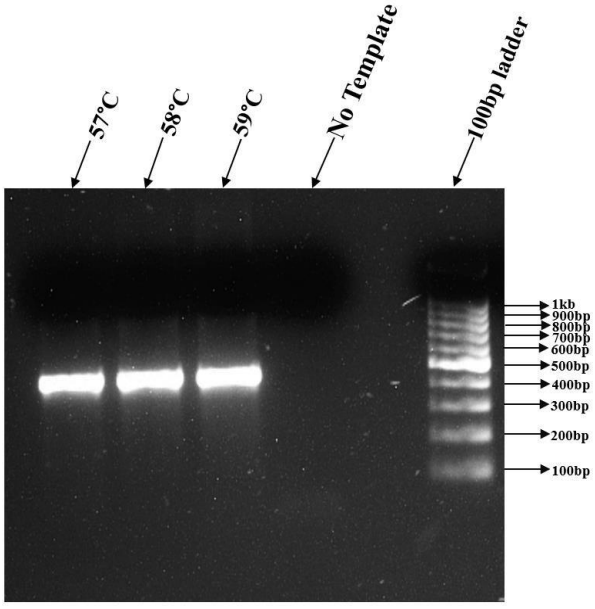


Fig – 02 [GRIN2B gradient PCR]

The sharpest band was observed at 59°C. Hence, we proceeded with that.

Each PCR reaction was set up in a total volume of 20 µL, containing:

- Genomic DNA – 1.75ul (35-40ng/ul)
- PCR Taq premix (2X) – 10ul
- Each primer – 0.3ul (16.67pmole/ul)
- Volume make up with nuclease-free water

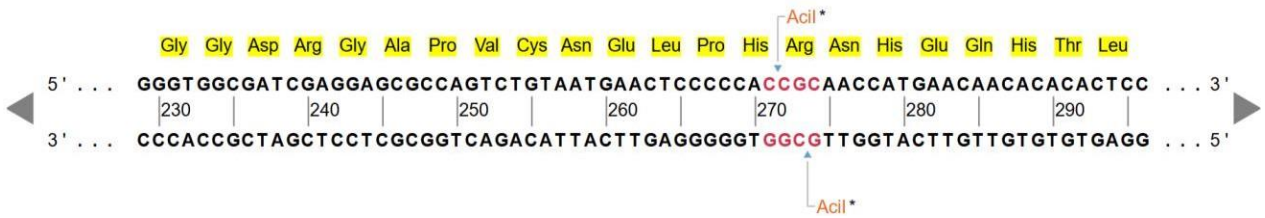
The PCR was performed in a thermal cycler with the following cycling conditions:

1. Initial Denaturation: 95°C for 5 minutes
2. Cycling Phase: (37 cycles)
    - Denaturation: 95°C for 30 seconds
    - Annealing: 59°C for 30 seconds
    - Extension: 72°C for 60 seconds
3. Final Extension: 72°C for 7 minutes
4. Final Hold: 4°C for ∞

The amplified products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination. A 100 bp DNA ladder was used as a molecular weight marker.

3.3.3 Restriction Fragment Length Polymorphism (RFLP) Analysis

The 466 bp PCR products were subjected to restriction digestion using AciI enzyme to detect polymorphisms in the GRIN2B variant. AciI is a tetra-cutter enzyme that recognizes the 5'-CCGC-3' sequence. The amplicon consisted of five AciI restriction sites with the **polymorphic site** being at **271bp** position (highlighted in the image attached) of the 466bp amplicon.


AciI*
Gly Gly Asp Arg Gly Ala Pro Val Cys Asn Glu Leu Pro His Arg Asn His Glu Gln His Thr Leu
5' . . . GGGTGGCGATCGAGGAGCGCCAGTCTGTAATGAACTCCCCCACCGCAACCATGAACAACACACACTCC . . . 3'
230 240 250 260 270 280 290
3' . . . CCCACCGCTAGCTCCTCGCGGTCAGACATTACTTGAGGGGGTGGCGTTGGTACTTGTTGTGTGTGAGG . . . 5'
AciI*

Ala Pro Ala Ala His Gly Gln Glu His Gly STP
AciI*
5' . . . CCTGCGCCTGCTGCGCACGGCCAAGAACATGGCTAACCTGTCTGGTGTGAATGGCTCACCGCAGAGCG . . . 3'
310 320 330 340 350 360
3' . . . GGACGCGGACGACGCGTGCCGGTTCTTGTACCGATTGGACAGACCACACTTACCGAGTGGCGTCTCGC . . . 5'
AciI*
AciI*
AciI*
AciI*
AciI*
5' . . . GTCTATGACATCTCAGAGCACCGCCGCAGCTTCACGCATTCTGACTGCAAATCCTACAACAACCCGCC . . . 3'
400 410 420 430 440 450 460
3' . . . CAGATACTGTAGAGTCTCGTGGCGGCGTCGAAGTGCGTAAGACTGACGTTTAGGATGTTGTTGGGCGG . . . 5'
AciI*
AciI*
AciI*
AciI*

Fig – 03 [AciI restriction sites]

[Source – NEBcutter 3.0]

The expected fragment sizes are as given below:

- Homozygous Wildtype (CC): 271 bp, 89 bp, 59bp, 40bp, 3bp
- Heterozygous (CT): 360 bp, 271 bp, 89 bp, 59bp, 40bp, 3bp
- Homozygous mutant (TT): 360 bp, 59 bp, 40bp, 3bp

Each RFLP reaction was set up in a total volume of 20 µL, containing:

- PCR product – 3ul
- CutSmart Buffer (10X) compatible with AciI enzyme – 2ul
- AciI enzyme (10,000 U/mL) – 0.3ul
- Nuclease-free water to make up the volume

The digestion mixture was incubated at 37°C for 1.5 hours.

3.3.4 Polyacrylamide Gel Electrophoresis (PAGE)

The digested products were run on a 7.5% native polyacrylamide gel to resolve the fragments generated by AciI digestion.

50 ml Gel Composition

- Acrylamide:Bisacrylamide (29:1) – 12.5ml
- TBE buffer (10X) – 5ml
- 20% Ammonium Persulfate (APS) – 200ul
- Freshly prepared TEMED – 200ul
- Volume makeup with distilled water

The gel was run at 100 volts for 2.5 hours at room temperature. After electrophoresis, the gel was kept dipped in water for 10 minutes for cooling the gel followed by staining with ethidium bromide for 10 minutes and visualized under UV light.

3.4 DRD4 variant

3.4.1 Primer Designing

Primers were designed with the help of NCBI primer blast tool to amplify a **408 bp** region containing the polymorphic site within the DRD4 variant genomic sequence.

Primers were designed to amplify a specific segment of the DRD4 gene containing the polymorphic site.

- Forward Primer: 5'-GGCCTCCCAGAAACAGACTA-3'
- Reverse Primer: 5'-CCGTTGCAGTTGATCCTC-3'

Fig – 04 [DRD4 PRIMERS]

[Source – SnapGene tool]

The melting temperature (Tm) of the primers were calculated by the standard 2 (A+T) + 4 (G+C) formula. The Tm of the DRD4 primers were calculated to be 62°C.

3.4.2 PCR Amplification

A specific segment of the DRD4 variant was amplified using PCR.

Firstly, three sets of PCR reactions were done in a gradient manner with annealing temperatures equal to or below the Tm = 62°C. The temperatures considered were 59°C, 60°C, 61°C and 62°C.

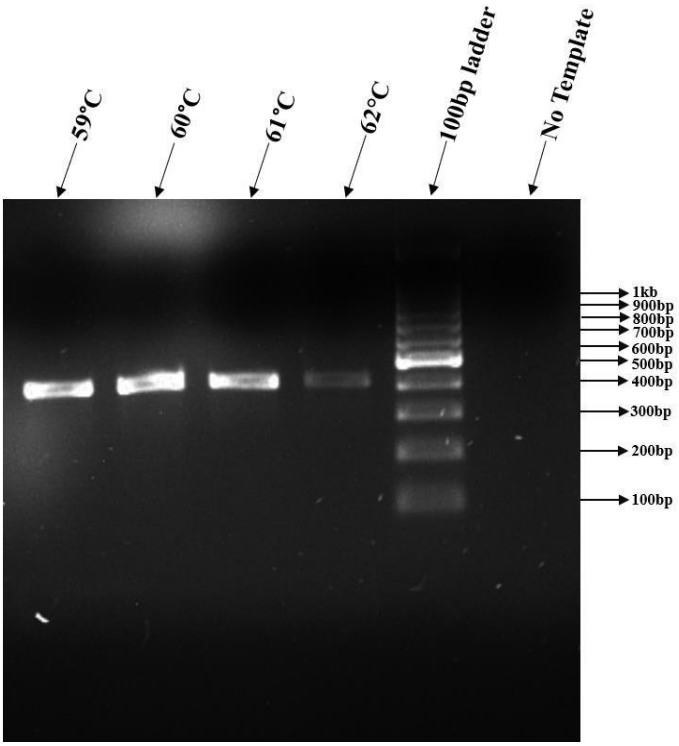


Fig – 05 [DRD4 gradient PCR]

The sharpest band was observed at 60°C. Hence, we proceeded with that.

Each PCR reaction was set up in a total volume of 20 µL, containing:

- Genomic DNA – 1.75ul (35-40ng/ul)
- PCR Taq premix (2X) – 10ul
- Each primer – 0.3ul (16.67pmole/ul)
- Volume make up with nuclease-free water

The PCR was performed in a thermal cycler with the following cycling conditions:

1. Initial Denaturation: 95°C for 5 minutes
2. Cycling Phase: (35 cycles)

- Denaturation: 95°C for 30 seconds
- Annealing: 60°C for 30 seconds
- Extension: 72°C for 60 seconds

3. Final Extension: 72°C for 7 minutes
4. Final Hold: 4°C for ∞

The amplified products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination. A 100 bp DNA ladder was used as a molecular weight marker.

3.4.3 Restriction Fragment Length Polymorphism (RFLP) Analysis

The 408 bp PCR products were subjected to restriction digestion using EcoRI enzyme to detect polymorphisms in the DRD4 gene. EcoRI is a hexa-cutter enzyme that recognizes the 5'-GAATTC-3' sequence. The 408bp amplicon consists of only one EcoRI restriction site that is the polymorphic site too at **155bp** position.

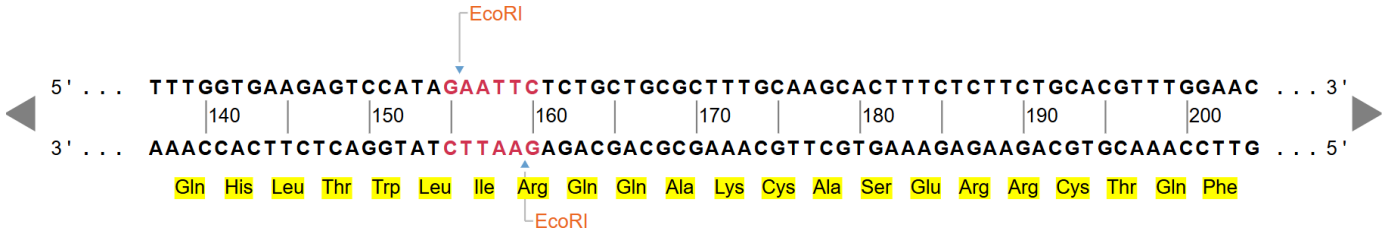

EcoRI
5' . . . TTTGGTGAAGAGTCCATAGAATTCTCTGCTGCGCTTTGCAAGCACTTTCTCTTCTGCACGTTTGGAAC . . . 3'
140
150
160
170
180
190
200
3' . . . AAACCACTTCTCAGGTATCTTAAGAGACGACGCGAAACGTTCGTGAAAGAGAAGACGTGCAAACCTTG . . . 5'
Gln His Leu Thr Trp Leu Ile Arg Gln Gln Ala Lys Cys Ala Ser Glu Arg Arg Cys Thr Gln Phe
EcoRI

Fig – 06 [EcoRI restriction site]

[Source – NEBcutter 3.0]

The expected fragment sizes are as given below:

- Homozygous Wildtype (TT): 253bp, 155bp
- Heterozygous (TC): 408bp, 253bp, 155bp
- Homozygous mutant (CC): 408bp

Each RFLP reaction was set up in a total volume of 20 µL, containing:

- PCR product – 3ul
- Buffer (10X) compatible with EcoRI enzyme – 2ul
- EcoRI enzyme (10,000 U/mL) – 0.4ul
- Nuclease-free water to make up the volume

The digestion mixture was incubated at 37°C for 4 hours.

3.4.4. Polyacrylamide Gel Electrophoresis (PAGE)

The digested products were run on a 7.5% native polyacrylamide gel to resolve the fragments generated by EcoRI digestion.

50 ml Gel Composition:

- Acrylamide:Bisacrylamide (29:1) – 12.5ml
- TBE buffer (10X) – 5ml
- 20% Ammonium Persulfate (APS) – 200ul
- Freshly prepared TEMED – 200ul
- Volume makeup with distilled water

The gel was run at 100 volts for 2.5 hours at room temperature. After electrophoresis, the gel was kept dipped in water for 10 minutes for the ease of removing the glass plates followed by staining with ethidium bromide for 10 minutes and visualized under UV light.

3.5 Genotyping and Distribution of SNPs

Genotyping was carried out by RFLP (Restriction Fragment Length Polymorphism) of the PCR amplified DNA samples. The allele and genotype frequencies for each SNP were determined and checked for conformity with Hardy–Weinberg Equilibrium (HWE) using SNPStats (https://www.snpstats.net/start.htm). Deviations from HWE may indicate genotyping errors, population stratification, or true biological effects. All SNPs considered met HWE expectations, validating the reliability of the genotypic data.

3.5.1. Statistical Analysis of Genotype–Phenotype Association

To explore the association between SNP genotypes and cognitive scores, data were analyzed using the SNPStats online tool, which allows for logistic and linear regression modeling of genotype–phenotype relationships. The cognitive scores (obtained using standardized neuropsychological tests) were treated as continuous variables, and genotypes were analyzed under different genetic models such as co-dominant, dominant, recessive, overdominant.

A confidence interval (CI) of 95% was applied, meaning we are 95% confident that the true value lies within this range. The alpha level (p-value threshold) was set at 0.05 (standard), meaning a p-value less than 0.05 indicates statistical significance i.e., the observed association is unlikely due to chance.

3.6. Indian Genetic Disease Database (IGDD): Data collection, curation, and arrangement of monogenic disorders

Here in this work, monogenic diseases, that is diseases for which a single unique gene is responsible were chosen. After choosing the monogenic diseases, we searched for research articles published with the data of Indian patients with those monogenic diseases. Data (mutation data, clinical data, etc.) were taken from those available papers.

Along with this the single gene (responsible for the disease) information was taken from OMIM (Online Mendelian Inheritance in Man), Genecard, KEGG (Kyoto Encyclopedia of Genes and Genomes), NCBI (National Center for Biotechnology Information) databases. The inheritance pattern of the diseases were also mentioned in OMIM.

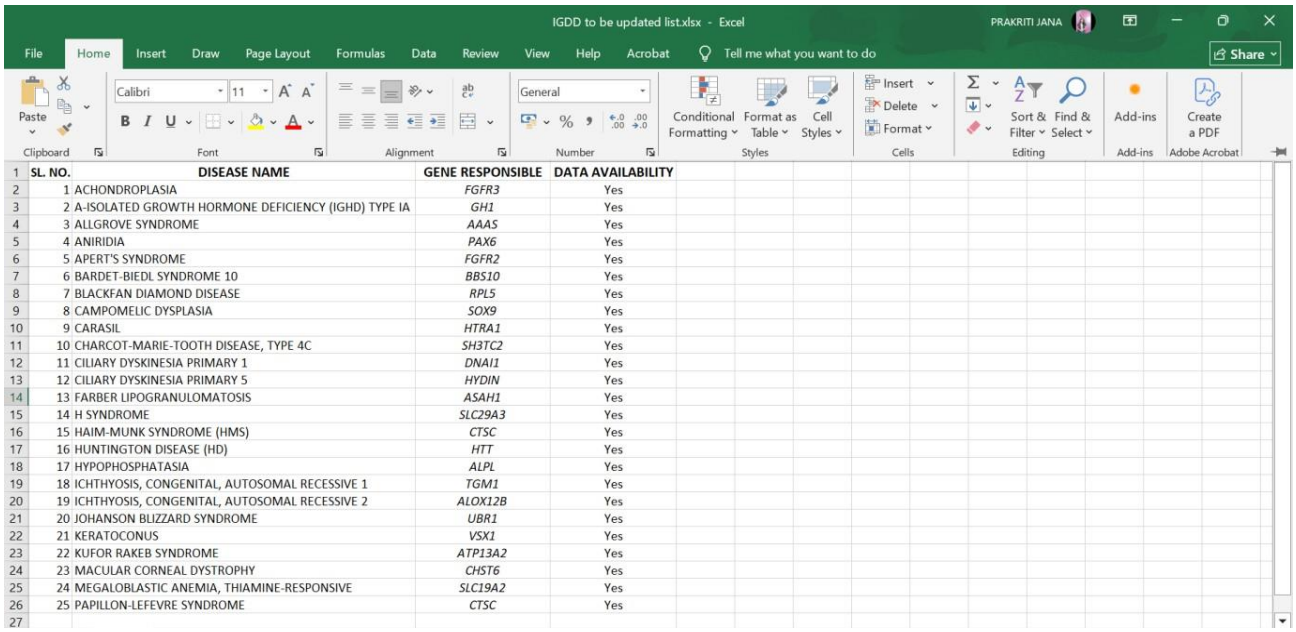
The disease information, gene information and inheritance information were documented in Excel sheet, and the overall information statistics are documented in another file (Word format). Another Word file contains some broad highlights about the disease. Thus, each disease contains three files for different ways of overall presentation.

3.6.1 OBJECTIVE OF THE STUDIES DONE FOR IGDD:

- To update 25 monogenic diseases by extracting information from relevant literature from NCBI on the gene responsible for the disease along with the type of mutation, inheritance pattern, etc as well as diagnostic tests to identify mutations and carriers of the disease.
- To update gene information for 8 monogenic diseases based on OMIM (Online Mendelian Inheritance in Man), Genecards, KEGG (Kyoto Encyclopedia of Genes and Genomes) databases.

3.6.2. PROCEDURE FOR DISEASE UPDATE

(1) Twenty-five Monogenic diseases were chosen based on their data availability.



IGDD to be updated list.xlsx - Excel

SL. NO.	DISEASE NAME	GENE RESPONSIBLE	DATA AVAILABILITY
1	ACHONDROPLASIA	*FGFR3*	Yes
2	A-ISOLATED GROWTH HORMONE DEFICIENCY (IGHD) TYPE IA	*GH1*	Yes
3	ALLGROVE SYNDROME	*AAAS*	Yes
4	ANIRIDIA	*PAX6*	Yes
5	APERT'S SYNDROME	*FGFR2*	Yes
6	BARDET-BIEDL SYNDROME 10	*BBS10*	Yes
7	BLACKFAN DIAMOND DISEASE	*RPL5*	Yes
8	CAMPOMELIC DYSPLASIA	*SOX9*	Yes
9	CARASIL	*HTRA1*	Yes
10	CHARCOT-MARIE-TOOTH DISEASE, TYPE 4C	*SH3TC2*	Yes
11	CILIARY DYSKINESIA PRIMARY 1	*DNAI1*	Yes
12	CILIARY DYSKINESIA PRIMARY 5	*HYDIN*	Yes
13	FARBER LIPOGRANULOMATOSIS	*ASAH1*	Yes
14	H SYNDROME	*SLC29A3*	Yes
15	HAIM-MUNK SYNDROME (HMS)	*CTSC*	Yes
16	HUNTINGTON DISEASE (HD)	*HTT*	Yes
17	HYPOPHOSPHATASIA	*ALPL*	Yes
18	ICHTHYOSIS, CONGENITAL, AUTOSOMAL RECESSIVE 1	*TGM1*	Yes
19	ICHTHYOSIS, CONGENITAL, AUTOSOMAL RECESSIVE 2	*ALOX12B*	Yes
20	JOHANSON BLIZZARD SYNDROME	*UBR1*	Yes
21	KERATOCONUS	*VSX1*	Yes
22	KUFOR RAKEB SYNDROME	*ATP13A2*	Yes
23	MACULAR CORNEAL DYSTROPHY	*CHST6*	Yes
24	MEGALOBLASTIC ANEMIA, THIAMINE-RESPONSIVE	*SLC19A2*	Yes
25	PAPILLON-LEFEVRE SYNDROME	*CTSC*	Yes

Fig – 07 (List of monogenic diseases)

(2) After choosing the monogenic diseases, research articles were investigated over the web and other data archive platforms like **PubMed**, which are published with the data of Indian patients affected with the selected monogenic diseases. In the PubMed website the disease name and gene name were typed in the search box in the following format "***Disease name and responsible gene name and mutation and India***" in order get more accurate results.

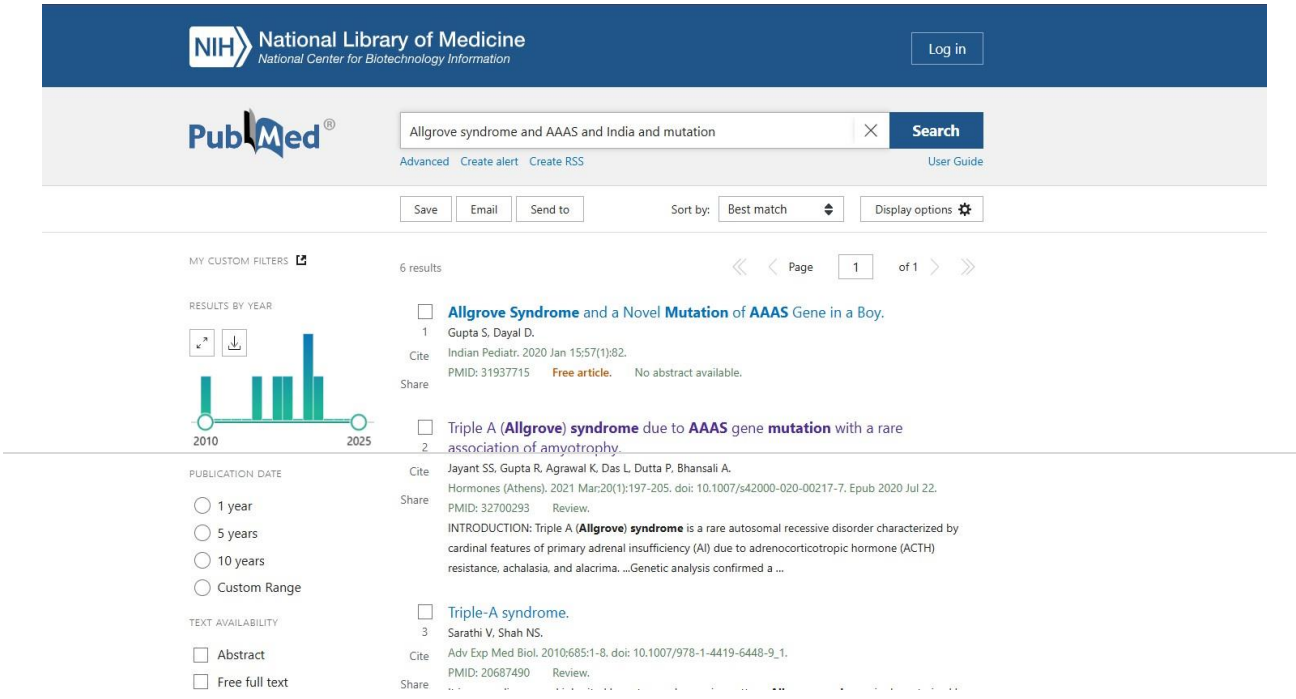

National Library of Medicine
National Center for Biotechnology Information
Log in
PubMed
Allgrove syndrome and AAAS and India and mutation
Search
Advanced
Create alert
Create RSS
User Guide
Save
Email
Send to
Sort by: Best match
Display options
MY CUSTOM FILTERS
6 results
Page 1 of 1
RESULTS BY YEAR
2010
2025
PUBLICATION DATE
1 year
5 years
10 years
Custom Range
TEXT AVAILABILITY
Abstract
Free full text
Allgrove Syndrome and a Novel Mutation of AAAS Gene in a Boy.
Gupta S, Dayal D.
Indian Pediatr. 2020 Jan 15;57(1):82.
PMID: 31937715 Free article. No abstract available.
Triple A (Allgrove) syndrome due to AAAS gene mutation with a rare association of amyotrophy.
Jayant SS, Gupta R, Agrawal K, Das L, Dutta P, Bhansali A.
Hormones (Athens). 2021 Mar;20(1):197-205. doi: 10.1007/s42000-020-00217-7. Epub 2020 Jul 22.
PMID: 32700293 Review.
INTRODUCTION: Triple A (Allgrove) syndrome is a rare autosomal recessive disorder characterized by cardinal features of primary adrenal insufficiency (AI) due to adrenocorticotropic hormone (ACTH) resistance, achalasia, and alacrima. ...Genetic analysis confirmed a ...
Triple-A syndrome.
Sarathi V, Shah NS.
Adv Exp Med Biol. 2010;685:1-8. doi: 10.1007/978-1-4419-6448-9_1.
PMID: 20687490 Review.

Fig – 08 (Image source: PubMed)

(3) Papers containing Indian patient cohort was taken.

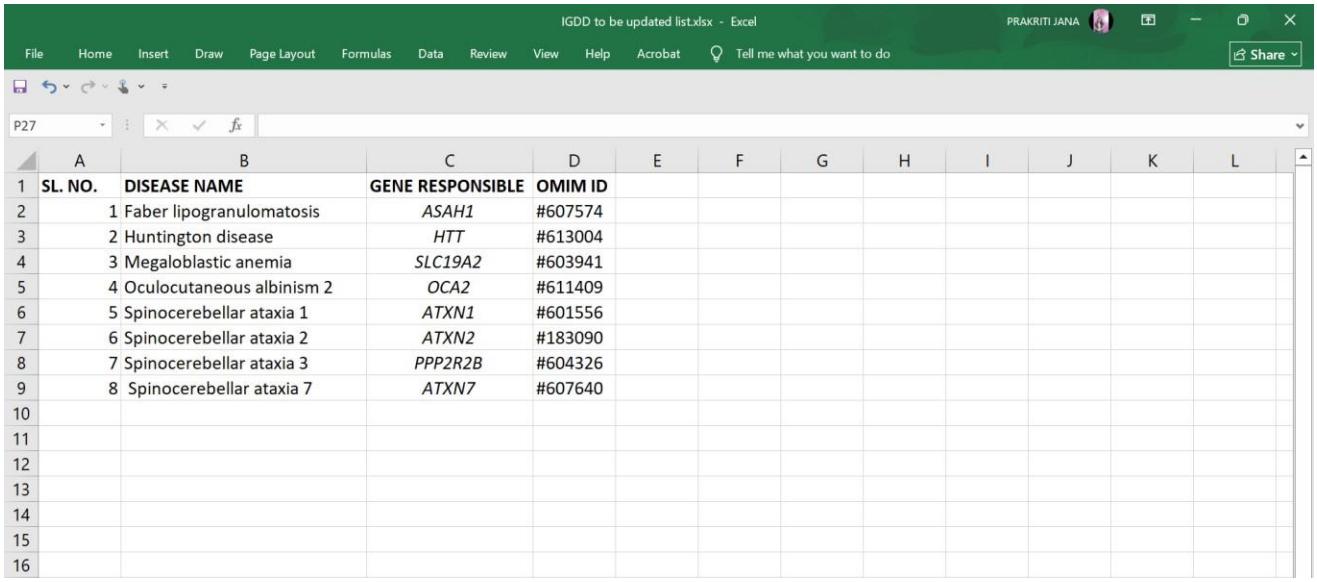
(4) Papers which fulfilled the above criteria but didn't have any proper information about patient's mutations (at least either nucleotide change or amino acid change must be presented) was not be taken.

(5) Review articles and papers with data which did not have any mutation observed via wet-lab procedures but by prediction via bioinformatics tool were not taken.

(6) Data was taken from the available papers which satisfied the above criterias.

3.6.3 PROCEDURE FOR GENE INFORMATION UPDATE

(1) Gene information was collected for eight monogenic diseases.


IGDD to be updated list.xlsx - Excel
PRAKRITI JANA
File Home Insert Draw Page Layout Formulas Data Review View Help Acrobat Tell me what you want to do
Share
P27

	SL. NO.	DISEASE NAME	GENE RESPONSIBLE	OMIM ID
1	1	Faber lipogranulomatosis	*ASAH1*	#607574
2	2	Huntington disease	*HTT*	#613004
3	3	Megaloblastic anemia	*SLC19A2*	#603941
4	4	Oculocutaneous albinism 2	*OCA2*	#611409
5	5	Spinocerebellar ataxia 1	*ATXN1*	#601556
6	6	Spinocerebellar ataxia 2	*ATXN2*	#183090
7	7	Spinocerebellar ataxia 3	*PPP2R2B*	#604326
8	8	Spinocerebellar ataxia 7	*ATXN7*	#607640

Fig – 09 (List diseases for which the responsible gene's information was collected)

(2) The gene information was collected from OMIM (Online Mendelian Inheritance in Man), Genecard and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases.

3.6.4 DATA ARRANGEMENT

1. The obtained disease information, gene information and inheritance information was documented in an excel sheet / spreadsheet and the overall information statistics was documented in another file (word format). Another word file was created which contained some broad highlights about the disease.
2. Thus, each of the 25 monogenic diseases contains three files for different way of overall presentation.

Sl. No.	TYPE OF DATA	FILE TYPE
1	• Disease Information • Gene Information • Inheritance Information • Mutation Data	EXCEL SHEET/ SPREADSHEET
2	Overview of entire analysis and statistical Data	STAT FILE
3	Broad Highlights about the disease	WORD FILE

Table – 01 [Type of Data and File Type]

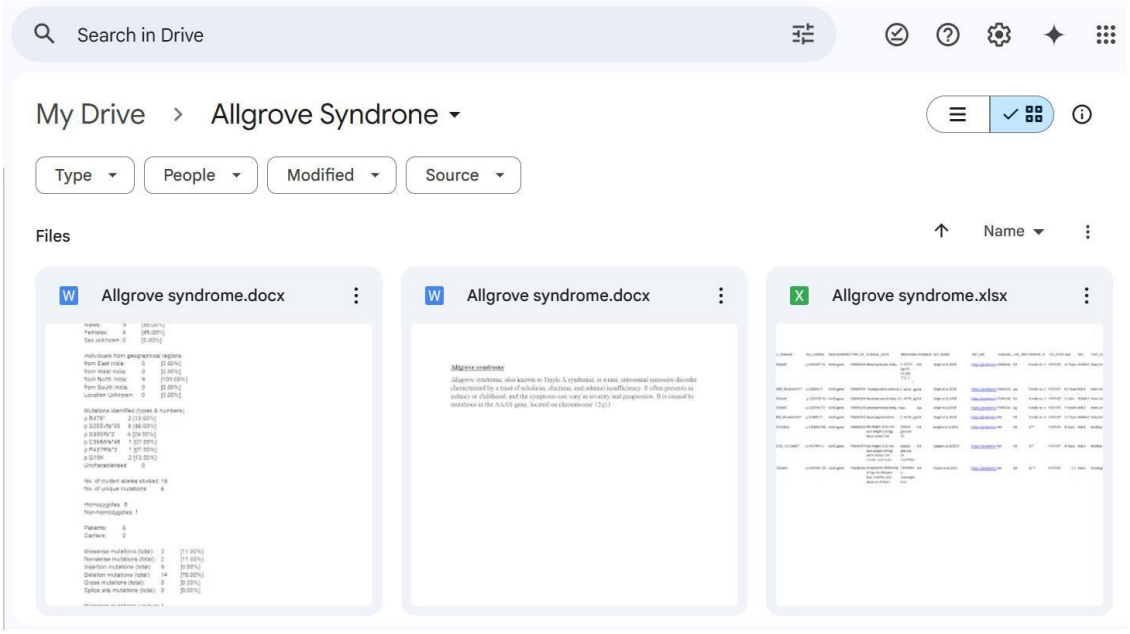


Fig – 10 [Types of files for data arrangement]

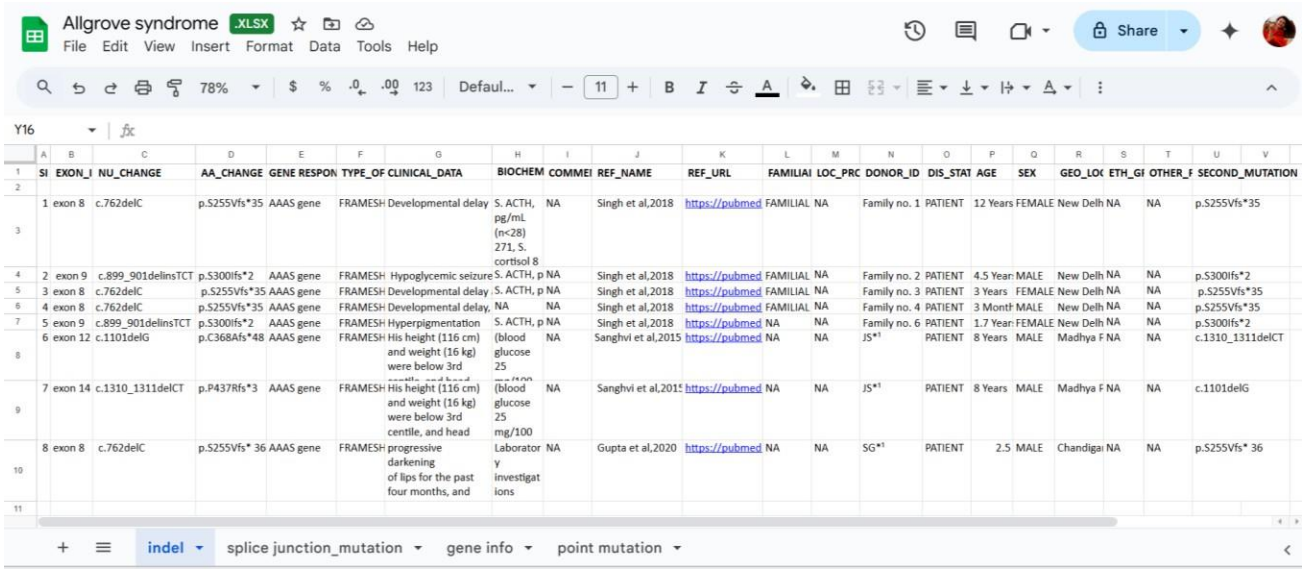
3. Each excel sheet for the disease was divided into **four pages,** the pages were named as **Indel, Splice site mutation, Gene info and Point mutation respectively.**

4. Each mutation page had the below written column names,

'EXON_INTRON', 'NU_CHANGE', 'AA_CHANGE', 'GENE RESPONSIBLE', 'TYPE_OF_MUTATION', 'CLINICAL_DATA', 'BIOCHEMICAL_DATA', 'COMMENTS', 'REF_NAME', 'REF_URL', 'FAMILIAL_SPORADIC', 'LOC_PROTEIN', 'DONOR_ID', 'DIS_STATUS', 'AGE', 'SEX', 'GEO_LOC', 'ETH_GR',

'OTHER_POPULATION', 'SECOND_MUTATION'.

5. After that, the mutational data from respective research articles were enlisted in the specific mutation type page.



SI	EXON_I	NU_CHANGE	AA_CHANGE	GENE RESPON	TYPE_OF	CLINICAL_DATA	BIOCHEM	COMMEI	REF_NAME	REF_URL	FAMILIAI	LOC_PRC	DONOR_ID	DIS_STAT	AGE	SEX	GEO_LOC	ETH_GI	OTHER_F	SECOND_MUTATION
1	exon 8	c.762delC	p.S255Vfs*35	AAAS gene	FRAMESH	Developmental delay	S. ACTH, pg/mL (n<28) 271, S. cortisol 8	NA	Singh et al,2018	https://pubmed	FAMILIAL	NA	Family no. 1	PATIENT	12 Years	FEMALE	New Delh	NA	NA	p.S255Vfs*35
2	exon 9	c.899_901delinsTCT	p.S300Ifs*2	AAAS gene	FRAMESH	Hypoglycemic seizure	S. ACTH, p	NA	Singh et al,2018	https://pubmed	FAMILIAL	NA	Family no. 2	PATIENT	4.5 Year	MALE	New Delh	NA	NA	p.S300Ifs*2
3	exon 8	c.762delC	p.S255Vfs*35	AAAS gene	FRAMESH	Developmental delay	S. ACTH, p	NA	Singh et al,2018	https://pubmed	FAMILIAL	NA	Family no. 3	PATIENT	3 Years	FEMALE	New Delh	NA	NA	p.S255Vfs*35
4	exon 8	c.762delC	p.S255Vfs*35	AAAS gene	FRAMESH	Developmental delay,	NA	NA	Singh et al,2018	https://pubmed	FAMILIAL	NA	Family no. 4	PATIENT	3 Month	MALE	New Delh	NA	NA	p.S255Vfs*35
5	exon 9	c.899_901delinsTCT	p.S300Ifs*2	AAAS gene	FRAMESH	Hyperpigmentation	S. ACTH, p	NA	Singh et al,2018	https://pubmed	NA	NA	Family no. 6	PATIENT	1.7 Year	FEMALE	New Delh	NA	NA	p.S300Ifs*2
6	exon 12	c.1101delG	p.C368Afs*48	AAAS gene	FRAMESH	His height (116 cm) and weight (16 kg) were below 3rd	(blood glucose 25	NA	Sanghvi et al,2015	https://pubmed	NA	NA	JS*1	PATIENT	8 Years	MALE	Madhya F	NA	NA	c.1310_1311delCT
7	exon 14	c.1310_1311delCT	p.P437Rfs*3	AAAS gene	FRAMESH	His height (116 cm) and weight (16 kg) were below 3rd centile, and head	(blood glucose 25 mg/100	NA	Sanghvi et al,201	https://pubmed	NA	NA	JS*1	PATIENT	8 Years	MALE	Madhya F	NA	NA	c.1101delG
8	exon 8	c.762delC	p.S255Vfs* 36	AAAS gene	FRAMESH	progressive darkening of lips for the past four months, and	Laboratory investigations	NA	Gupta et al,2020	https://pubmed	NA	NA	SG*1	PATIENT	2.5	MALE	Chandiga	NA	NA	p.S255Vfs* 36

Fig – 11 [Data arrangement in Excel/Spreadsheet]

6. For the ref name the 'last name of the first author of the article' along with et al, and year of publishing the paper was noted.

7. If the donor ID was not mentioned in the paper, then the donor ID was written as the initials of the first author along with the no. of the patient.

8. Finally, all the disease information, gene information and inheritance information were documented in an excel sheet and the overall information statistics of the obtained information was documented in another file. Another word file was created in which some broad highlights about the disease was written. Thus, each disease contains three files for different way of overall presentation.

Disease name: Allgrove syndrome

No. of individuals studied: 9

Males: 5 [55.00%]
Females: 4 [45.00%]
Sex unknown: 0 [0.00%]

Individuals from geographical regions:
from East India: 0 [0.00%]
from West India: 0 [0.00%]
from North India: 9 [100.00%]
from South India: 0 [0.00%]
Location Unknown: 0 [0.00%]

Mutations identified (types & numbers):
p.R478* 2 [13.00%]
p.S255Vfs*35 8 [46.00%]
p.S300Ifs*2 4 [24.00%]
p.C368Afs*48 1 [07.00%]
p.P437Rfs*3 1 [07.00%]
p.G15K 2 [13.00%]
Uncharacterised 0

No. of mutant alleles studied: 18
No. of unique mutations: 6

Homozygotes: 8
Non-homozygotes: 1

Patients: 9
Carriers: 0

Missense mutations (total): 2 [11.00%]
Nonsense mutations (total): 2 [11.00%]
Insertion mutations (total): 0 [0.00%]
Deletion mutations (total): 14 [78.00%]
Gross mutations (total): 0 [0.00%]
Splice site mutations (total): 0 [0.00%]

Missense mutations (unique): 1
Nonsense mutations (unique): 1
Insertion mutations (unique): 0
Deletion mutations (unique): 0
Gross mutations (unique): 4
Splice site mutations (unique): 0

Fig - 12 [Stat File containing the overview of entire analysis and statistical Data]

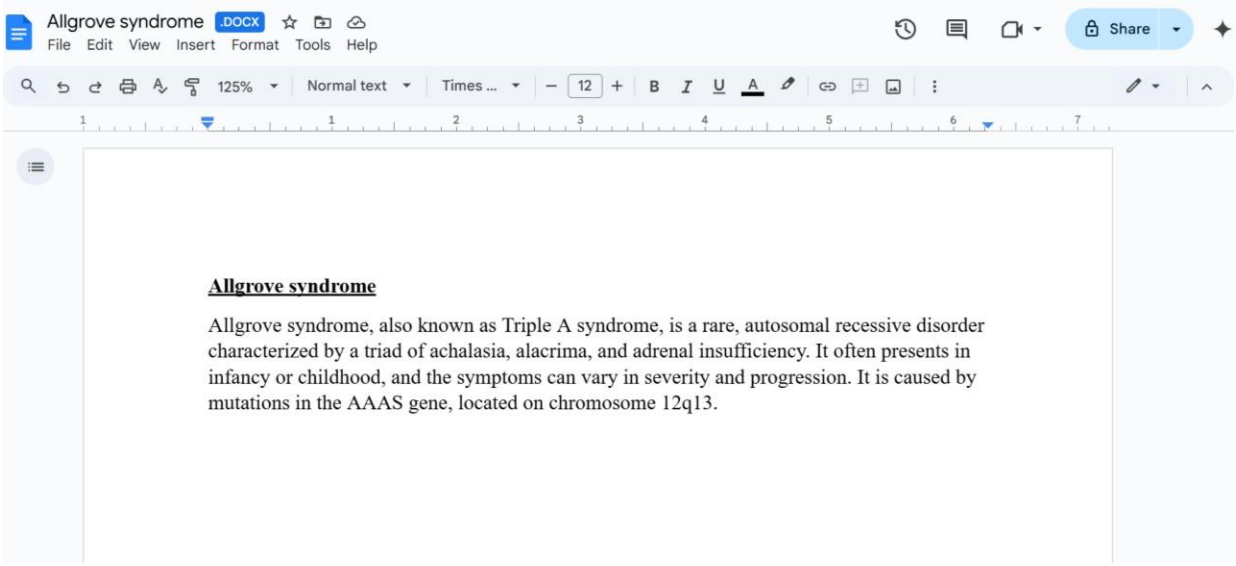

Allgrove syndrome .DOCX
File Edit View Insert Format Tools Help
Share
125%
Normal text
Times ...
12
Allgrove syndrome
Allgrove syndrome, also known as Triple A syndrome, is a rare, autosomal recessive disorder characterized by a triad of achalasia, alacrima, and adrenal insufficiency. It often presents in infancy or childhood, and the symptoms can vary in severity and progression. It is caused by mutations in the AAAS gene, located on chromosome 12q13.

Fig – 13 [Word File containing broad Highlights about the disease]

9. For the gene info we added the following columns in the excel sheet:
'DIS_NAME', 'GENE_NAME', 'CHR_LOC', 'NU_LEN', 'AA_LEN', 'NU_ACC', 'PRO_ACC', 'NU_URL', 'AA_URL', 'GENE_CARD_LINK', 'OMIM_GENEMAP_LINK',' 'UNIPROT_url', 'KEGG_url', 'PDB_ID', 'PDB_URL', 'ENTR_ID', 'ENTR_URL', 'UNIPROT_ID', 'KEGG_ID', 'GENE_OMIM_ID', 'GENE_OMIM_URL', 'MODE_INHERITANCE', 'ABBREVIATIONS' in the first row.

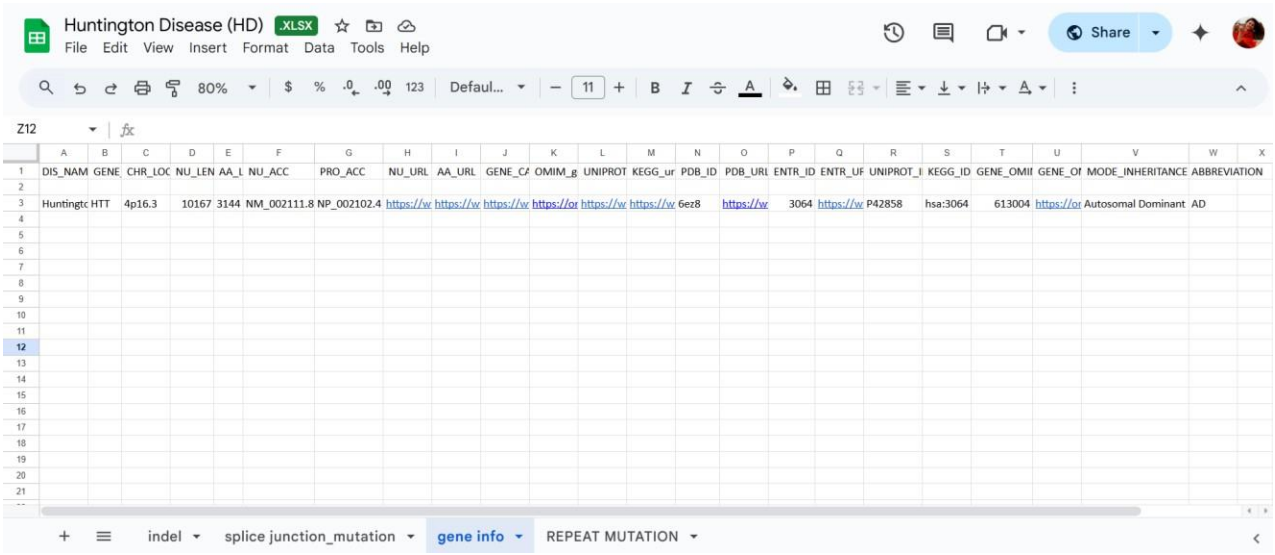

Huntington Disease (HD)
.XLSX
File Edit View Insert Format Data Tools Help
Share
Z12
DIS_NAM GENE CHR_LOC NU_LEN AA_L NU_ACC PRO_ACC NU_URL AA_URL GENE_CA OMIM_g UNIPROT KEGG_ur PDB_ID PDB_URL ENTR_ID ENTR_UF UNIPROT_I KEGG_ID GENE_OMII GENE_OI MODE_INHERITANCE ABBREVIATION
Huntingtc HTT 4p16.3 10167 3144 NM_002111.8 NP_002102.4 https://w https://w https://w https://or https://w https://w 6ez8 https://w 3064 https://w P42858 hsa:3064 613004 https://or Autosomal Dominant AD
indel
splice junction_mutation
gene info
REPEAT MUTATION

Fig – 14 [Data arrangement on gene information page]

10. The information of single gene responsible for the disease was collected from the omim (Online Mendelian Inheritance in Man), Genecards, KEGG (Kyoto Encyclopedia of Genes and Genomes) databases. The NCBI was searched in the browser and then we went to 'OMIM.' In the OMIM database the responsible gene name was investigated to get all necessary data. Genecards, Uniport, KEGG were also found in the OMIM.

4. RESULTS AND DISCUSSIONS

4.1. GRIN2B VARIANT

4.1.1. PCR RESULTS

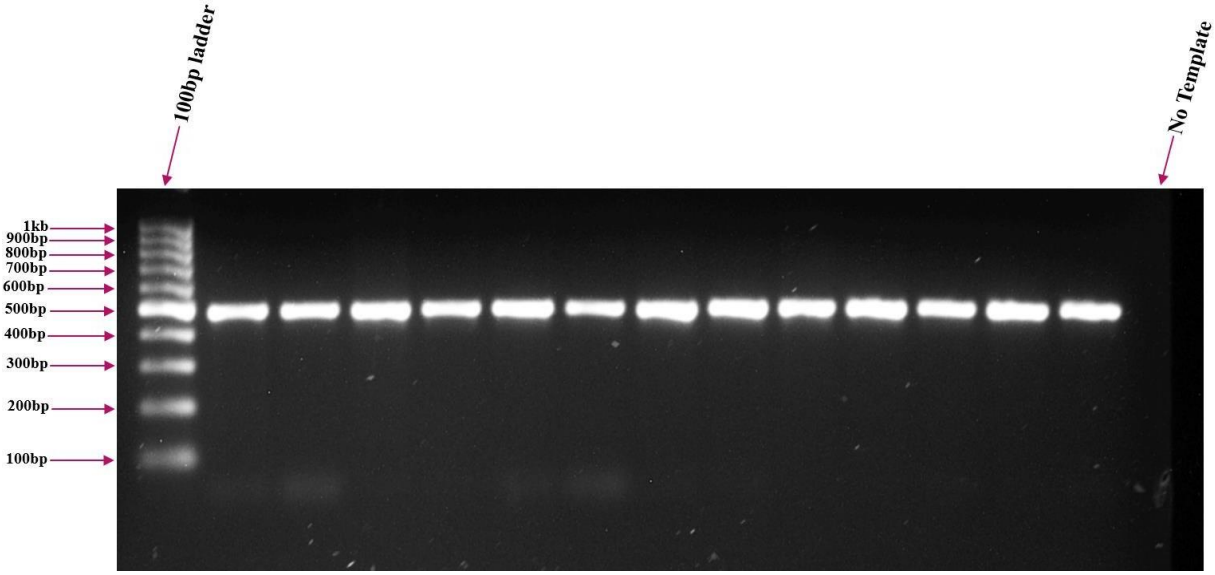


Fig – 15 [GRIN2B PCR products run on 1.5% agarose gel]

As expected, sharp bands at 466bp were observed.

4.1.2 RFLP RESULTS

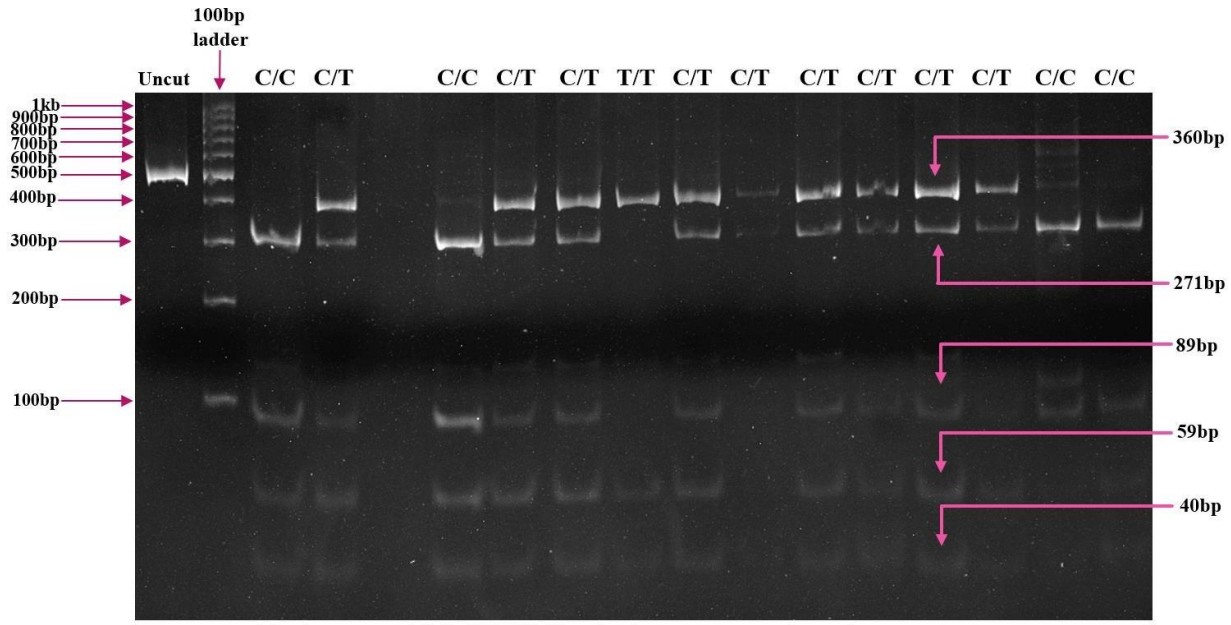


Fig – 16 [GRIN2B RFLP products run on 7.5% acrylamide gel]

Genotype Interpretation

The genotypes were interpretated based on the digestion pattern (fragment sizes).

The expected fragment sizes for each genotype were:

- Homozygous Wildtype (CC): 271 bp, 89 bp, 59bp, 40bp, 3bp
- Heterozygous (CT): 360 bp, 271 bp, 89 bp, 59bp, 40bp, 3bp
- Homozygous mutant (TT): 360 bp, 59 bp, 40bp, 3bp

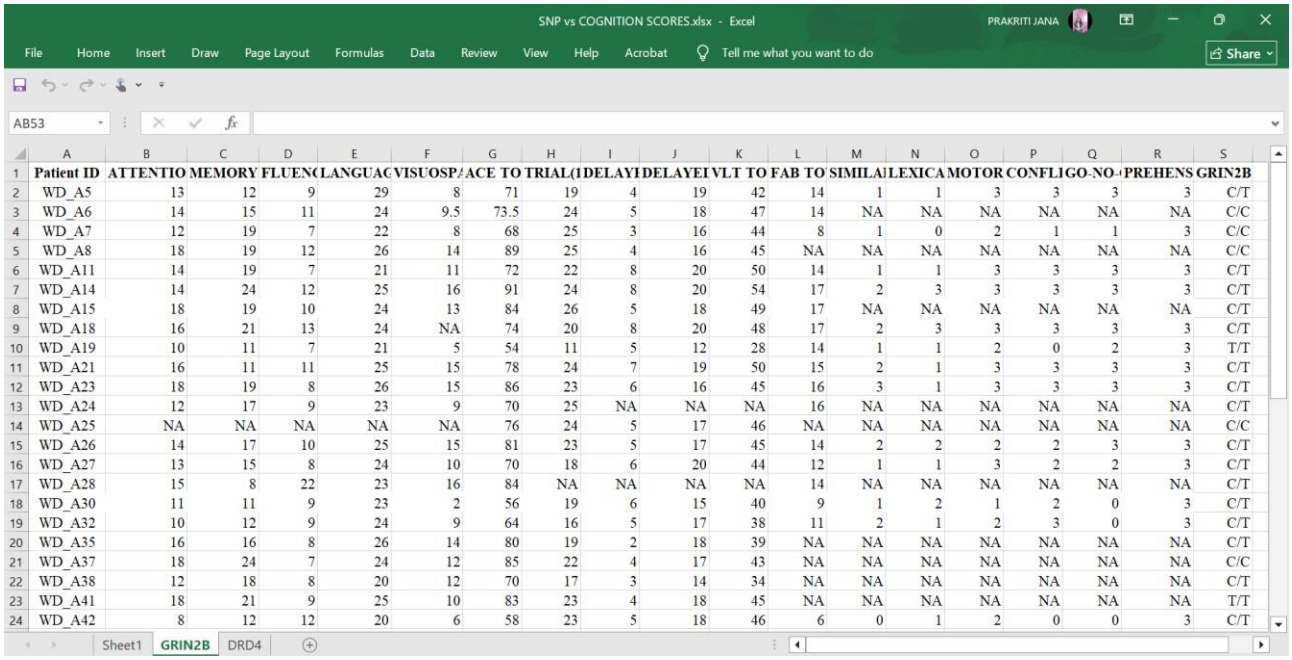
Fragment sizes were compared with molecular weight markers to confirm the genotypes.

4.1.3 GENOTYPING

SL. NO.	PATIENT ID	GENOTYPE	SL. NO.	PATIENT ID	GENOTYPE
1	WD_5	C/T	24	WD_45	C/T
2	WD_6	C/C	25	WD_46	T/T
3	WD_7	C/C	26	WD_50	C/T
4	WD_8	C/C	27	WD_52	C/T
5	WD_11	C/T	28	WD_54	C/T
6	WD_14	C/T	29	WD_55	C/T
7	WD_15	C/T	30	WD_58	C/C
8	WD_18	C/T	31	WD_61	C/C
9	WD_19	T/T	32	WD_65	C/T
10	WD_21	C/T	33	WD_68	C/T
11	WD_23	C/T	34	WD_73	C/T
12	WD_24	C/T	35	WD_74	T/T
13	WD_25	C/C	36	WD_78	C/C
14	WD_26	C/T	37	WD_79	T/T
15	WD_27	C/T	38	WD_80	C/C
16	WD_28	C/T	39	WD_82	C/T
17	WD_30	C/T	40	WD_84	T/T
18	WD_32	C/T	41	WD_85	C/T
19	WD_35	C/T	42	WD_92	C/C
20	WD_37	C/C	43	WD_93	C/C
21	WD_38	C/T	44	WD_97	C/C
22	WD_41	T/T	45	WD_98	C/T
23	WD_42	C/T			

Table – 02 [45 Indian Wilson disease patient samples were genotyped and analyzed]

4.1.4. GRIN2B vs COGNITION SCORES



SNP vs COGNITION SCORES.xlsx - Excel

Patient ID	ATTENTIO	MEMORY	FLUEN	LANGUAG	VISUOSP	ACE TO	TRIAL(I	DELAYI	DELAYEI	VLT TO	FAB TO	SIMILA	LEXICA	MOTOR	CONFLI	GO-NO-	PREHENS	GRIN2B
WD_A5	13	12	9	29	8	71	19	4	19	42	14	1	1	3	3	3	3	C/T
WD_A6	14	15	11	24	9.5	73.5	24	5	18	47	14	NA	NA	NA	NA	NA	NA	C/C
WD_A7	12	19	7	22	8	68	25	3	16	44	8	1	0	2	1	1	3	C/C
WD_A8	18	19	12	26	14	89	25	4	16	45	NA	NA	NA	NA	NA	NA	NA	C/C
WD_A11	14	19	7	21	11	72	22	8	20	50	14	1	1	3	3	3	3	C/T
WD_A14	14	24	12	25	16	91	24	8	20	54	17	2	3	3	3	3	3	C/T
WD_A15	18	19	10	24	13	84	26	5	18	49	17	NA	NA	NA	NA	NA	NA	C/T
WD_A18	16	21	13	24	NA	74	20	8	20	48	17	2	3	3	3	3	3	C/T
WD_A19	10	11	7	21	5	54	11	5	12	28	14	1	1	2	0	2	3	T/T
WD_A21	16	11	11	25	15	78	24	7	19	50	15	2	1	3	3	3	3	C/T
WD_A23	18	19	8	26	15	86	23	6	16	45	16	3	1	3	3	3	3	C/T
WD_A24	12	17	9	23	9	70	25	NA	NA	NA	16	NA	NA	NA	NA	NA	NA	C/T
WD_A25	NA	NA	NA	NA	NA	76	24	5	17	46	NA	NA	NA	NA	NA	NA	NA	C/C
WD_A26	14	17	10	25	15	81	23	5	17	45	14	2	2	2	2	3	3	C/T
WD_A27	13	15	8	24	10	70	18	6	20	44	12	1	1	3	2	2	3	C/T
WD_A28	15	8	22	23	16	84	NA	NA	NA	NA	14	NA	NA	NA	NA	NA	NA	C/T
WD_A30	11	11	9	23	2	56	19	6	15	40	9	1	2	1	2	0	3	C/T
WD_A32	10	12	9	24	9	64	16	5	17	38	11	2	1	2	3	0	3	C/T
WD_A35	16	16	8	26	14	80	19	2	18	39	NA	NA	NA	NA	NA	NA	NA	C/T
WD_A37	18	24	7	24	12	85	22	4	17	43	NA	NA	NA	NA	NA	NA	NA	C/C
WD_A38	12	18	8	20	12	70	17	3	14	34	NA	NA	NA	NA	NA	NA	NA	C/T
WD_A41	18	21	9	25	10	83	23	4	18	45	NA	NA	NA	NA	NA	NA	NA	T/T
WD_A42	8	12	12	20	6	58	23	5	18	46	6	0	1	2	0	0	3	C/T

Sheet1 | GRIN2B | DRD4

Fig – 17 [GRIN2B vs COGNITION SCORES OF WD PATIENTS]

4.1.5. Regression analysis of GRIN2B variant

(a) Lexical Fluency

GRIN2B association with response LEXICAL.FLUENCY.FAB. (n=45, crude analysis)

Model	Genotype	n	Response mean (s.e.)	Difference (95% CI)	P-value	AIC	BIC
Codominant	C/C	7	0.71 (0.29)	0.00			
	C/T	20	1.45 (0.18)	**0.74 (0.06 - 1.41)**	0.12	77.6	83.3
	T/T	4	1.25 (0.25)	0.54 (-0.43 - 1.50)			
Dominant	C/C	7	0.71 (0.29)	0.00			
	C/T-T/T	24	1.42 (0.16)	**0.70 (0.05 - 1.35)**	0.043	75.8	80.1
Recessive	C/C-C/T	27	1.26 (0.17)	0.00			
	T/T	4	1.25 (0.25)	-0.01 (-0.88 - 0.86)	0.98	80.3	84.6
Overdominant	C/C-T/T	11	0.91 (0.21)	0.00			
	C/T	20	1.45 (0.18)	0.54 (-0.04 - 1.12)	0.077	76.9	81.2
Log-additive	---	---	---	0.35 (-0.13 - 0.83)	0.16	78.1	82.4

Fig - 18 [GRIN2B vs Lexical Fluency]

Dominant model is significant (p = 0.043)

Interpretation: C/T and T/T genotypes perform better than C/C in the dominant model as they have higher response mean = 1.42 than the C/C genotype.

(b) Conflicting Instructions

GRIN2B association with response CONFLICTING.INSTRUCTION.FAB. (n=45, crude analysis)							
Model	Genotype	n	Response mean (s.e.)	Difference (95% CI)	P-value	AIC	BIC
Codominant	C/C	7	2.14 (0.26)	0.00	0.066	88.8	94.6
	C/T	20	2.25 (0.2)	0.11 (-0.70 - 0.91)			
	T/T	4	1 (0.71)	-1.14 (-2.29 - 0.01)			
Dominant	C/C	7	2.14 (0.26)	0.00	0.82	92.8	97.1
	C/T-T/T	24	2.04 (0.22)	-0.10 (-0.95 - 0.75)			
Recessive	C/C-C/T	27	2.22 (0.16)	0.00	0.02	86.9	91.2
	T/T	4	1 (0.71)	**-1.22 (-2.19 - -0.25)**			
Overdominant	C/C-T/T	11	1.73 (0.33)	0.00	0.17	90.7	95
	C/T	20	2.25 (0.2)	0.52 (-0.20 - 1.24)			
Log-additive	---	---	---	-0.45 (-1.03 - 0.14)	0.14	90.5	94.8

Fig - 19 [GRIN2B vs Conflicting instructions]

Recessive model is significant (p = 0.02). The **T/T genotype** shows the lowest performance with Response Mean = 1.

Interpretation: The T/T individuals in the recessive model had greater difficulty in resolving and responding to conflicting stimuli, than the C/C + C/T individuals.

(c) TRIAL 1.1.1

GRIN2B association with response TRIAL.1.1.1..VLT. (n=45, crude analysis)							
Model	Genotype	n	Response mean (s.e.)	Difference (95% CI)	P-value	AIC	BIC
Codominant	C/C	12	20.25 (1.27)	0.00	0.11	252.5	259.6
	C/T	25	20 (0.7)	-0.25 (-3.22 - 2.72)			
	T/T	6	16 (2.8)	**-4.25 (-8.47 - -0.03)**			
Dominant	C/C	12	20.25 (1.27)	0.00	0.5	254.8	260.1
	C/T-T/T	31	19.23 (0.81)	-1.02 (-4.00 - 1.96)			
Recessive	C/C-C/T	37	20.08 (0.62)	0.00	0.035	250.5	255.8
	T/T	6	16 (2.8)	**-4.08 (-7.75 - -0.41)**			
Overdominant	C/C-T/T	18	18.83 (1.31)	0.00	0.4	254.5	259.8
	C/T	25	20 (0.7)	1.17 (-1.53 - 3.87)			
Log-additive	---	---	---	-1.74 (-3.80 - 0.32)	0.1	252.5	257.7

Fig - 20 [GRIN2B vs Lexical Fluency]

Recessive model (p = 0.035) is statistically significant. Individuals with T/T genotype had the lowest recall scores with a response mean = 16, while C/T + C/C scored higher (response mean 20.08).

Interpretation: T allele in homozygous state (T/T genotype) appears to be detrimental for immediate memory, possibly due to altered NMDA receptor function affecting synaptic encoding.

4.2. DRD4 VARIANT

4.2.1 PCR RESULTS

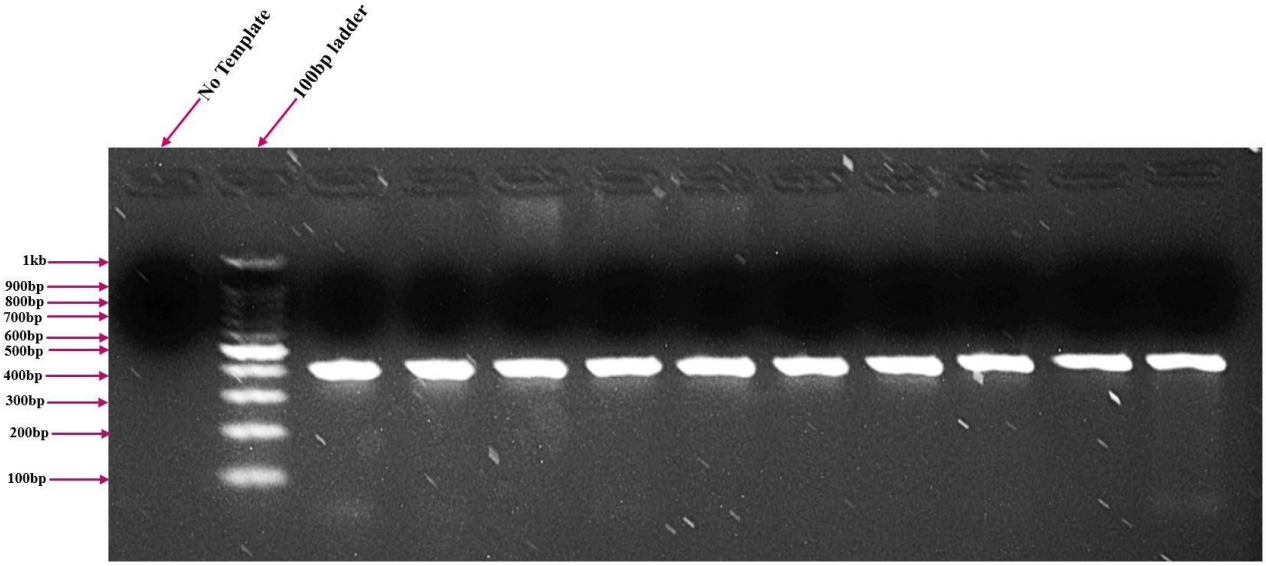


Fig – 21 [DRD4 PCR products run on 1.5% agarose gel]

As expected, sharp bands at 408bp were observed.

4.2.2 RFLP RESULTS

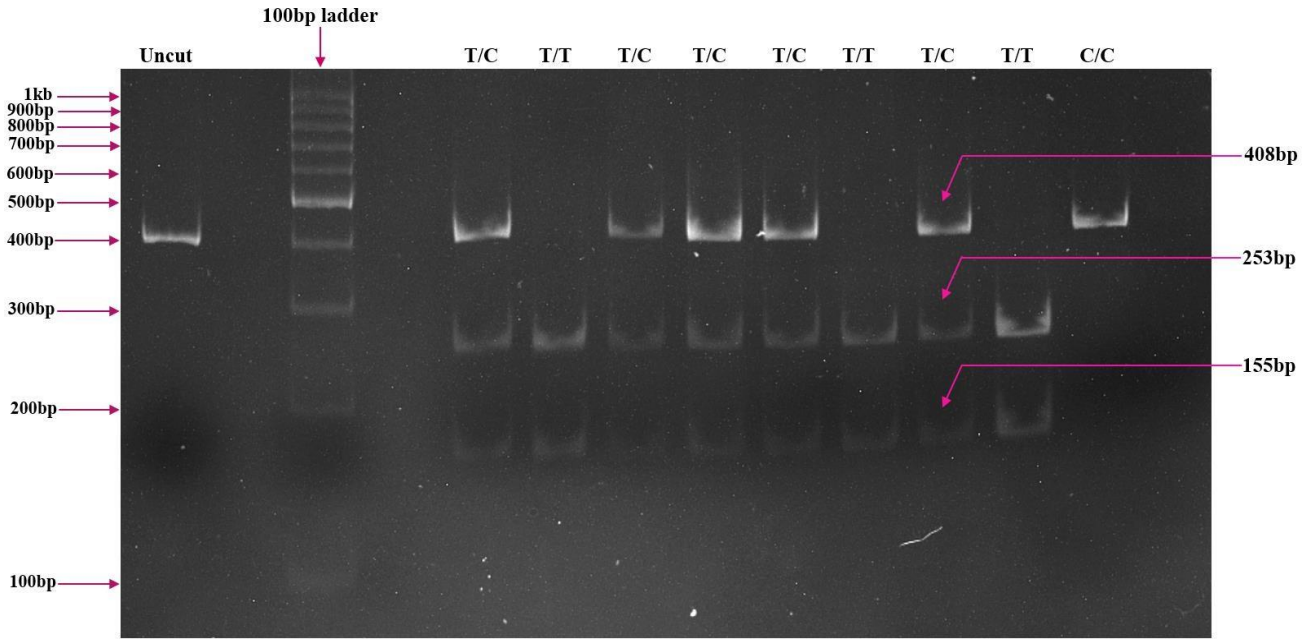


Fig – 22 [DRD4 RFLP products run on 7.5% acrylamide gel]

Genotype Interpretation

The genotypes were interpretated based on the digestion pattern (fragment sizes).

The expected fragment sizes for each genotype were:

- Homozygous Wildtype (CC): 253bp, 155bp
- Heterozygous (CT): 408bp, 253bp, 155bp
- Homozygous mutant (TT): 408bp

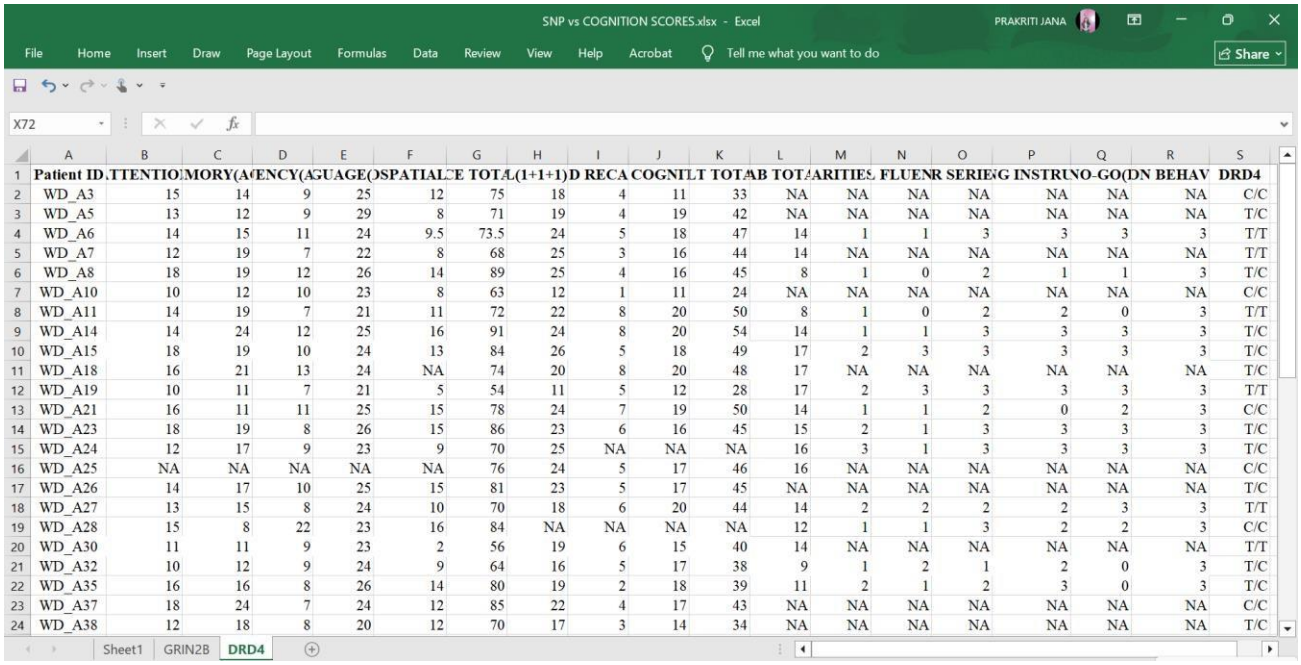
Fragment sizes were compared with molecular weight markers to confirm the genotypes.

4.2.3 GENOTYPING

SL. NO.	PATIENT ID	GENOTYPE	SL. NO.	PATIENT ID	GENOTYPE
1	WD_3	C/C	26	WD_43	C/C
2	WD_5	T/C	27	WD_45	T/C
3	WD_6	T/T	28	WD_46	C/C
4	WD_7	T/T	29	WD_50	T/T
5	WD_8	T/C	30	WD_52	T/C
6	WD_10	C/C	31	WD_54	T/C
7	WD_11	T/T	32	WD_55	T/C
8	WD_14	T/C	33	WD_58	T/T
9	WD_15	T/C	34	WD_59	C/C
10	WD_18	T/C	35	WD_60	T/T
11	WD_19	T/T	36	WD_61	T/C
12	WD_21	C/C	37	WD_65	C/C
13	WD_23	T/C	38	WD_68	T/C
14	WD_24	T/C	39	WD_73	T/C
15	WD_25	C/C	40	WD_74	C/C
16	WD_26	T/C	41	WD_78	T/C
17	WD_27	T/T	42	WD_79	T/T
18	WD_28	C/C	43	WD_80	T/C
19	WD_30	T/T	44	WD_82	T/C
20	WD_32	T/C	45	WD_84	T/C
21	WD_35	T/C	46	WD_85	T/C
22	WD_37	C/C	47	WD_92	T/T
23	WD_38	T/C	48	WD_93	T/C
24	WD_41	T/C	49	WD_97	T/C
25	WD_42	T/T	50	WD_98	T/T

Table – 03 [50 Indian Wilson disease patient samples were genotyped and analyzed]

4.2.4. DRD4 vs COGNITION SCORES



SNP vs COGNITION SCORES.xlsx - Excel

Patient ID	ATTENTIO	MORY(A	ENCY(A	GUAGE	OSPATIAL	E TOTA	(1+1+1)	D RECA	COGNTL	T TOTA	AB TOTA	ARITIES	FLUENR	SERIE	G INSTRU	NO-GO(D	N BEHAV	DRD4
WD_A3	15	14	9	25	12	75	18	4	11	33	NA	NA	NA	NA	NA	NA	NA	C/C
WD_A5	13	12	9	29	8	71	19	4	19	42	NA	NA	NA	NA	NA	NA	NA	T/C
WD_A6	14	15	11	24	9.5	73.5	24	5	18	47	14	1	1	3	3	3	3	T/T
WD_A7	12	19	7	22	8	68	25	3	16	44	14	NA	NA	NA	NA	NA	NA	T/T
WD_A8	18	19	12	26	14	89	25	4	16	45	8	1	0	2	1	1	3	T/C
WD_A10	10	12	10	23	8	63	12	1	11	24	NA	NA	NA	NA	NA	NA	NA	C/C
WD_A11	14	19	7	21	11	72	22	8	20	50	8	1	0	2	2	0	3	T/T
WD_A14	14	24	12	25	16	91	24	8	20	54	14	1	1	3	3	3	3	T/C
WD_A15	18	19	10	24	13	84	26	5	18	49	17	2	3	3	3	3	3	T/C
WD_A18	16	21	13	24	NA	74	20	8	20	48	17	NA	NA	NA	NA	NA	NA	T/C
WD_A19	10	11	7	21	5	54	11	5	12	28	17	2	3	3	3	3	3	T/T
WD_A21	16	11	11	25	15	78	24	7	19	50	14	1	1	2	0	2	3	C/C
WD_A23	18	19	8	26	15	86	23	6	16	45	15	2	1	3	3	3	3	T/C
WD_A24	12	17	9	23	9	70	25	NA	NA	NA	16	3	1	3	3	3	3	T/C
WD_A25	NA	NA	NA	NA	NA	76	24	5	17	46	16	NA	NA	NA	NA	NA	NA	C/C
WD_A26	14	17	10	25	15	81	23	5	17	45	NA	NA	NA	NA	NA	NA	NA	T/C
WD_A27	13	15	8	24	10	70	18	6	20	44	14	2	2	2	2	3	3	T/T
WD_A28	15	8	22	23	16	84	NA	NA	NA	NA	12	1	1	3	2	2	3	C/C
WD_A30	11	11	9	23	2	56	19	6	15	40	14	NA	NA	NA	NA	NA	NA	T/T
WD_A32	10	12	9	24	9	64	16	5	17	38	9	1	2	1	2	0	3	T/C
WD_A35	16	16	8	26	14	80	19	2	18	39	11	2	1	2	3	0	3	T/C
WD_A37	18	24	7	24	12	85	22	4	17	43	NA	NA	NA	NA	NA	NA	NA	C/C
WD_A38	12	18	8	20	12	70	17	3	14	34	NA	NA	NA	NA	NA	NA	NA	T/C

Sheet1 GRIN2B DRD4

Fig – 23 [DRD4 vs COGNITION SCORES OF WD PATIENTS]

4.2.5. Regression analysis of DRD4 variant

(a) Fluency ACE

DRD4 association with response FLUENCY.ACE. (n=50, crude analysis)						
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	T/T	13	8.15 (0.53)	0.00			
	T/C	26	9.58 (0.34)	1.42 (-0.22 - 3.07)	0.077	232.6	240.1
	C/C	10	10.5 (1.34)	**2.35 (0.31 - 4.38)**			
Dominant	T/T	13	8.15 (0.53)	0.00	0.041	231.6	237.3
	T/C-C/C	36	9.83 (0.44)	**1.68 (0.11 - 3.25)**			
Recessive	T/T-T/C	39	9.1 (0.3)	0.00	0.12	233.5	239.2
	C/C	10	10.5 (1.34)	1.40 (-0.35 - 3.15)			
Overdominant	T/T-C/C	23	9.17 (0.68)	0.00	0.59	235.7	241.4
	T/C	26	9.58 (0.34)	0.40 (-1.04 - 1.85)			
Log-additive	---	---	---	**1.19 (0.19 - 2.19)**	0.024	230.7	236.4

Fig - 24 [DRD4 vs Fluency ACE]

Dominant model shows significant difference (p = 0.041). T/C and C/C carriers had significantly higher fluency scores than T/T.

Interpretation: T/C + C/C genotype has better fluency than T/T genotype. Also, the presence of at least one C allele (dominant model) significantly improves verbal fluency in WD patients. This could reflect enhanced dopaminergic function.

(b) Visuospatial ACE

DRD4 association with response VISUOSPATIAL.ACE. (n=50, crude analysis)							
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	T/T	13	8.81 (0.9)	0.00			
	T/C	25	11.34 (0.63)	**2.53 (0.24 - 4.83)**	0.061	259.3	266.8
	C/C	10	11.85 (1.33)	**3.04 (0.22 - 5.87)**			
Dominant	T/T	13	8.81 (0.9)	0.00	0.019	257.5	263.1
	T/C-C/C	35	11.49 (0.58)	**2.68 (0.52 - 4.84)**			
Recessive	T/T-T/C	38	10.47 (0.55)	0.00	0.28	262.1	267.7
	C/C	10	11.85 (1.33)	1.38 (-1.10 - 3.86)			
Overdominant	T/T-C/C	23	10.13 (0.82)	0.00	0.24	261.9	267.5
	T/C	25	11.34 (0.63)	1.21 (-0.80 - 3.22)			
Log-additive	---	---	---	**1.59 (0.18 - 3.00)**	0.032	258.4	264

Fig - 25 [DRD4 vs Visuospatial ACE]

Dominant model (p = 0.019) indicates significant association. T/C and C/C combined response mean = 11.49 is higher than T/T genotype.

Interpretation: T/C + C/C genotype show significantly better visuospatial function than T/T genotype.

(c) Delayed Cognition

DRD4 association with response DELAYED.COGNITION.VLT. (n=50, crude analysis)							
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	T/T	13	17.62 (0.73)	0.00			
	T/C	24	17.46 (0.64)	-0.16 (-2.71 - 2.40)	0.094	263.5	270.9
	C/C	10	14.5 (1.89)	-3.12 (-6.24 - 0.01)			
Dominant	T/T	13	17.62 (0.73)	0.00	0.43	265.9	271.4
	T/C-C/C	34	16.59 (0.74)	-1.03 (-3.54 - 1.48)			
Recessive	T/T-T/C	37	17.51 (0.48)	0.00	0.029	261.5	267.1
	C/C	10	14.5 (1.89)	**-3.01 (-5.63 - -0.40)**			
Overdominant	T/T-C/C	23	16.26 (0.95)	0.00	0.3	265.4	271
	T/C	24	17.46 (0.64)	1.20 (-1.04 - 3.43)			
Log-additive	---	---	---	-1.46 (-3.03 - 0.10)	0.073	263.2	268.7

Fig - 26 [DRD4 vs Delayed Cognition]

Recessive model is significant (p = 0.029), with C/C homozygote's mean score is lower than T/T + T/C genotype.

Interpretation: Unlike other domains of DRD4 variant, homozygous C/C genotype is associated with poorer delayed cognition. This contrasts with its positive role in fluency and visuospatial tasks.

(d) ACE total

DRD4 association with response ACE.TOTAL (n=50, crude analysis)							
Model	**Genotype**	**n**	**Response mean (s.e.)**	**Difference (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	T/T	13	65.04 (2.1)	0.00			
	T/C	26	73.1 (2.44)	**8.06 (0.74 - 15.38)**	0.026	386.5	394.2
	C/C	11	77.14 (3.17)	**12.10 (3.27 - 20.92)**			
Dominant	T/T	13	65.04 (2.1)	0.00	0.012	385.6	391.3
	T/C-C/C	37	74.3 (1.96)	**9.26 (2.31 - 16.21)**			
Recessive	T/T-T/C	39	70.41 (1.86)	0.00	0.09	389.2	395
	C/C	11	77.14 (3.17)	6.73 (-0.90 - 14.36)			
Overdominant	T/T-C/C	24	70.58 (2.2)	0.00	0.45	391.6	397.4
	T/C	26	73.1 (2.44)	2.51 (-3.97 - 8.99)			
Log-additive	---	---	---	**6.14 (1.76 - 10.51)**	0.0084	384.9	390.7

Fig - 27 [DRD4 vs ACE total]

Both Codominant (p = 0.026), **Dominant** (p = 0.012) **models**, both show strong significance.

T/C + C/C genotype performed better than T/T genotype in Dominant model. While in Codominant model C/C performed better than both T/T and T/C genotypes.

Interpretation: In DRD4 variant, C allele is significantly associated with improved global cognitive performance. This supports the hypothesis that DRD4 acts as a cognitive modifier gene in Wilson disease.

4.3 Indian Genetic Disease Database (IGDD)

- 25 monogenic diseases were shortlisted based on availability of data and their disease information were updated.

Table – 04 [Population Data of the 25 monogenic diseases are represented]

SL. NO.	NAME OF THE DISEASE	GENE RESPONSIBLE	NO. OF INDIVIDUALS STUDIED	GENDER			GEOGRAPHICAL LOCATION IN INDIA			
				M	F	O	NORTH	SOUTH	EAST	WEST
1	ACHONDROPLASIA	*FGFR3*	88	11	6	7	72	1	0	15
2	ISOLATED GROWTH HORMONE DEFICIENCY (IGHD) TYPE IA	*GH1*	17	9	8	0	0	3	5	9
3	ALLGROVE SYNDROME	*AAAS*	9	5	4	0	9	0	0	0
4	ANIRIDIA	*PAX6*	25	16	9	0	7	17	0	1
5	APERT'S SYNDROME	*FGFR2*	6	4	2	0	4	0	0	2
6	BARDET-BIEDL SYNDROME 10	*BBS10*	23	1	1	21	0	23	0	0
7	BLACKFAN DIAMOND DISEASE	*RPL5*	4	2	1	1	3	0	0	1
8	CAMPOMELIC DYSPLASIA	*SOX9*	1	1	0	0	0	1	0	0
9	CARASIL	*HTRA1*	3	2	1	0	0	3	0	0
10	CHARCOT-MARIE-TOOTH DISEASE, TYPE 4C	*SH3TC2*	10	2	3	5	0	5	0	5
11	CILIARY DYSKINESIA PRIMARY 1	*DNAI1*	2	1	1	0	2	0	0	0
12	CILIARY DYSKINESIA PRIMARY 5	*HYDIN*	6	2	4	0	4	2	0	0

13	FARBER LIPOGRANULOMATOSIS	*ASAH1*	16	4	11	1	0	12	0	4
14	H SYNDROME	*SLC29A3*	6	5	1	0	0	4	0	2
15	HAIM-MUNK SYNDROME (HMS)	*CTSC*	2	0	2	0	0	2	0	0
16	HUNTINGTON DISEASE (HD)	*HTT*	75	52	23	0	30	45	0	0
17	HYPOPHOSPHATASIA	*ALPL*	4	1	1	2	4	0	0	0
18	ICHTHYOSIS, CONGENITAL, AUTOSOMAL RECESSIVE 1	*TGM1*	14	4	4	6	0	6	4	4
19	ICHTHYOSIS, CONGENITAL, AUTOSOMAL RECESSIVE 2	*ALOX12B*	8	3	1	4	2	6	0	0
20	JOHANSON BLIZZARD SYNDROME	*UBR1*	2	1	1	0	1	0	0	1
21	KERATOCONUS	*VSX1*	20	9	2	9	3	12	5	0
22	KUFOR RAKEB SYNDROME	*ATP13A2*	4	3	1	0	0	3	1	0
23	MACULAR CORNEAL DYSTROPHY	*CHST6*	260	6	4	250	10	250	0	0
24	MEGALOBLASTIC ANEMIA, THIAMINE-RESPONSIVE	*SLC19A2*	4	1	3	0	0	3	0	1
25	PAPILLON-LEFEVRE SYNDROME	*CTSC*	12	8	4	0	0	8	0	4

Table – 05 [The no. of different types of mutation of the selected 25 diseases are given]

SL. NO.	NAME OF THE DISEASES	GENE RESPONSIBLE	PATIENT	CARRIER	MUTANT ALLELES STUDIED	UNIQUE MUTATIONS	HOMOZYGOTES	NON HOMOZYGOTES	TYPES OF MUTATIONS													
									MISSENSE		NONSENSE		INSERTION		DELETION		DUPLICATION		GROSS		SPLICE	
									T	U	T	U	T	U	T	U	T	U	T	U	T	U
1	ACHONDROPLASIA	*FGFR3*	88	0	88	2	0	88	88	2	0	0	0	0	0	0	0	0	0	0	0	0
2	A-ISOLATED GROWTH HORMONE DEFICIENCY (IGHD) TYPE IA	*GH1*	13	4	26	5	9	8	0	0	0	0	0	0	0	0	0	0	25	4	1	1
3	ALLGROVE SYNDROME	*AAAS*	9	0	18	6	8	1	2	1	2	1	0	0	14	4	0	0	0	0	0	0
4	ANIRIDIA	*PAX6*	25	25	15	0	25	0	0	2	2	12	7	4	0	0	0	0	0	0	7	4
5	APERT'S SYNDROME	*FGFR2*	6	0	6	2	0	6	6	2	0	0	0	0	0	0	0	0	0	0	0	0
6	BARDET-BIEDL SYNDROME 10	*BBS10*	23	0	32	14	6	17	12	4	2	2	0	0	18	8	0	0	0	0	0	0
7	BLACKFAN DIAMOND DISEASE	*RPL5*	4	0	4	4	0	4	2	2	1	1	0	0	1	1	0	0	0	0	0	0
8	CAMPOMELIC DYSPLASIA	*SOX9*	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
9	CARASIL	*HTRA1*	3	0	6	3	0	3	0	0	2	1	0	0	4	2	0	0	0	0	0	0
10	CHARCOT-MARIE-TOOTH DISEASE, TYPE 4C	*SH3TC2*	9	1	19	12	6	4	6	5	7	4	0	0	4	2	0	0	0	0	2	1
11	CILIARY DYSKINESIA PRIMARY 1	*DNAI1*	2	0	4	2	4	0	4	2	0	0	0	0	0	0	0	0	0	0	0	0
12	CILIARY DYSKINESIA PRIMARY 5	*HYDIN*	6	0	10	5	4	2	0	0	8	3	1	1	0	0	0	0	0	0	1	1

13	FARBER LIPOGRANULOMATOSIS	*ASAH1*	10	6	32	16	5	11	21	12	0	0	0	0	1	1	0	0	0	0	0	0
14	H SYNDROME	*SLC29A3*	6	0	12	4	6	0	6	2	4	1	0	0	2	1	0	0	0	0	0	0
15	HAIM-MUNK SYNDROME (HMS)	*CTSC*	2	0	4	2	2	0	2	1	2	1	0	0	0	0	0	0	0	0	0	0
16	HUNTINGTON DISEASE (HD)	*HTT*	75	0	75	2	75	0	0	0	0	0	0	0	0	0	75	2	0	0	0	0
17	HYPOPHOSPHATASIA	*ALPL*	4	0	4	2	0	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0
18	ICHTHYOSIS, CONGENITAL AUTOSOMAL RECESSIVE 1	*TGM1*	12	2	26	13	8	6	23	11	0	0	0	0	1	1	0	0	0	0	1	1
19	ICHTHYOSIS, CONGENITAL AUTOSOMAL RECESSIVE 2	*ALOX12B*	8	0	15	8	6	2	8	4	1	1	0	0	6	3	0	0	0	0	0	0
20	JOHANSON BLIZZARD SYNDROME	*UBR1*	2	0	4	3	1	1	2	1	0	0	0	0	0	0	0	0	0	0	2	2
21	KERATOCONUS	*VSX1*	20	0	20	10	0	20	15	7	0	0	0	0	0	0	0	0	0	0	5	3
22	KUFOR RAKEB SYNDROME	*ATP13A2*	4	0	7	5	2	2	5	4	2	1	0	0	0	0	0	0	0	0	0	0
23	MACULAR CORNEAL DYSTROPHY	*CHST6*	256	4	399	116	138	118	223	74	18	13	58	12	100	17	0	0	0	0	0	0
24	MEGALOBLASTIC ANEMIA THIAMINE-RESPONSIVE	*SLC19A2*	4	0	7	4	3	1	2	1	2	1	0	0	3	2	0	0	0	0	0	0
25	PAPILLON-LEFEVRE SYNDROME	*CTSC*	12	0	24	6	11	1	1	1	15	3	0	0	8	2	0	0	0	0	0	0

From excel sheet and the stat file that we have updated, we can conclude some points regarding the shortlisted diseases:

1. From the Populational Data, mutation is mostly concentrated in **South India (65%)**, then in **North India (24%)**, followed by **West India (8%)** and least concentrated in **East India (3%).**

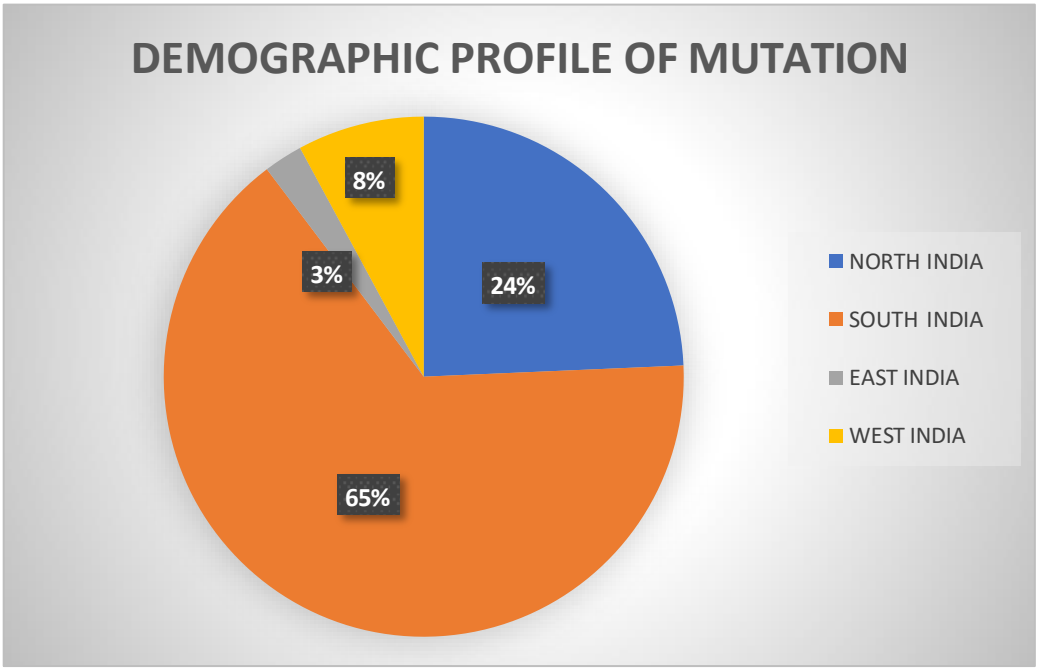


Fig - 28 [DEMOGRAPHIC PROFILE OF MUTATION]

2. **Males (27%)** are more susceptible to mutations than **females (18%)** according to the data available. It is hard to get into a fixed conclusion as in most data the gender was unknown (**55%)**.

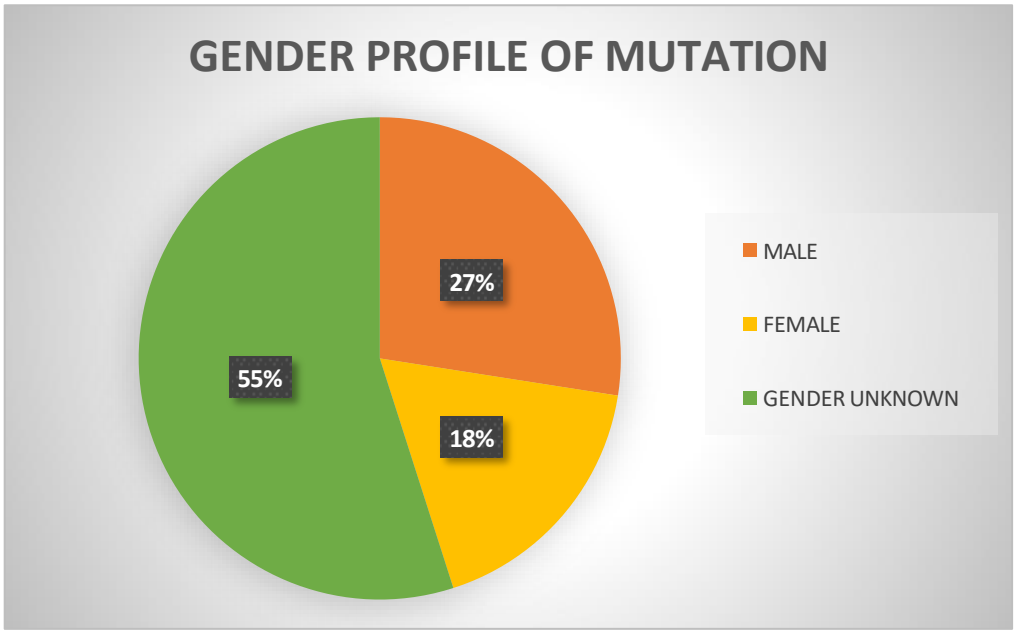


Fig - 29 [GENDER PROFILE OF MUTATION]

3. The ratio of percentage of total no. of mutations to unique mutations was 78:22.

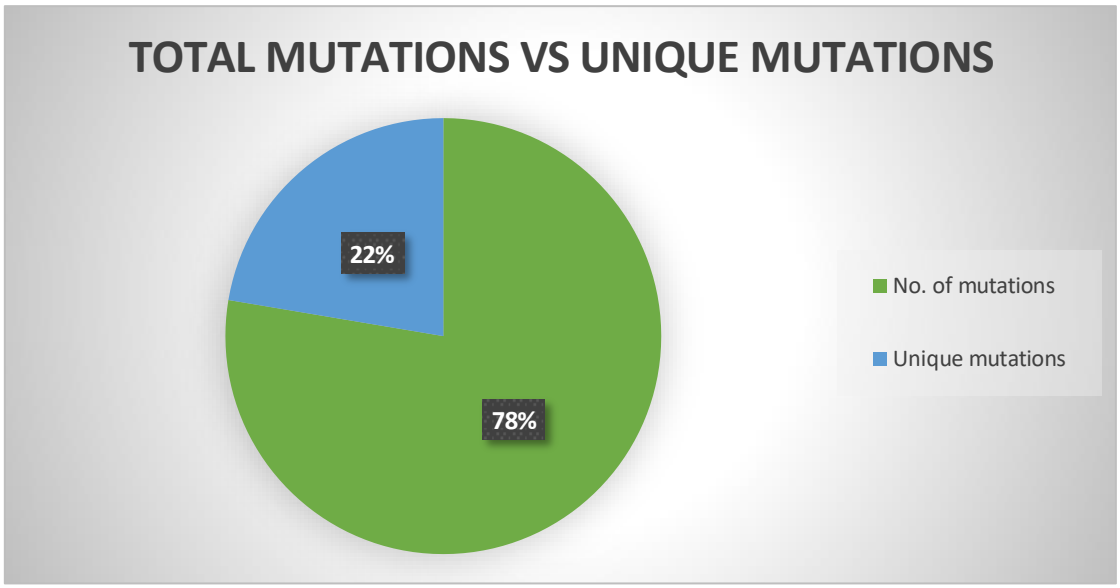


Fig - 30 [TOTAL MUTATIONS VS UNIQUE MUTATIONS]

5. CONCLUSION

5.1 GRIN2B variant

Trait	Genotype	SNP	Model	P value	Consequence	P-α value
Trial 1.1.1 VLT	T/T	GRIN2B	Recessive	0.035	Risk genotype	0.05
Lexical fluency FAB	C/T + T/T	GRIN2B	Dominant	0.043	Protective genotype	0.05
Conflicting instruction	T/T	GRIN2B	Recessive	0.02	Risk genotype	0.05

Table – 06 [Association of GRIN2B variant with cognition scores of Wilson Disease patients]

The findings of this study indicate that polymorphisms in the GRIN2B variant are significantly associated with specific cognitive traits in Indian Wilson disease patients. Among the cognitive domains analyzed, three showed statistically significant associations with GRIN2B genotypes ($p < 0.05$), supporting the gene's role as a potential cognitive modifier.

Notably, the T/T genotype under the recessive model was significantly associated with lower performance in immediate recall (Trial 1.1.1 VLT) and Conflicting instruction task, identifying it as a risk genotype for these traits. These results suggest that individuals homozygous for the T allele may have impaired NMDA receptor function, which could negatively affect memory consolidation and conflict resolution, cognitive functions heavily reliant on glutamatergic signalling.

Conversely, in the Lexical Fluency (FAB) domain, the C/T and T/T genotypes combined (dominant model) were associated with better verbal fluency scores, designating them as protective genotypes. This indicates a domain-specific beneficial effect of the T allele when present in at least one copy, possibly enhancing NMDA receptor activity relevant to language and fluency tasks.

Together, these results demonstrate that GRIN2B genotypes influence cognitive performance in a domain-dependent and genotype-specific manner. The T allele acts as a risk factor in homozygous state for memory and executive function, but shows a protective role in heterozygous form for language fluency. These findings underscore the complex role of GRIN2B in neurocognitive processing and support its consideration as a genetic modifier gene in Wilson disease.

Further investigation in larger cohorts, along with functional studies, is warranted to validate these associations and elucidate the underlying biological mechanisms.

5.2 DRD4 variant

Trait	Genotype	SNP	Model	P value	Consequence	p-α value
Fluency ACE	T/C + C/C	DRD4	Dominant	0.041	Protective genotype	0.05
Visuospatial ACE	T/C + C/C	DRD4	Dominant	0.019	Protective genotype	0.05
ACE total	C/C	DRD4	Codominant	0.026	Protective genotype	0.05
ACE total	T/C + C/C	DRD4	Dominant	0.012	Protective genotype	0.05
Delayed cognition	C/C	DRD4	Recessive	0.029	Risk genotype	0.05

Table – 07 [Association of DRD4 variant with cognition scores of Wilson Disease patients]

This study revealed significant associations between *DRD4* gene polymorphisms and multiple cognitive domains in Indian Wilson disease patients, supporting the role of *DRD4* as a potential cognitive modifier gene.

The dominant model of *DRD4* (T/C + C/C genotypes) was found to be significantly associated with improved performance in verbal fluency ($p = 0.041$), visuospatial ability ($p = 0.019$), and overall cognitive scores (ACE Total, $p = 0.012$). Similarly, the C/C genotype alone under the codominant model also showed a positive association with higher ACE total scores ($p = 0.026$). These findings consistently identify the C allele—particularly when present in at least one copy—as a protective genotype, possibly enhancing dopamine receptor function in neural circuits involved in executive and spatial processing.

However, under the recessive model, the C/C genotype was associated with poorer performance in delayed cognition ($p = 0.029$), suggesting a domain-specific risk effect when the allele is homozygous. This duality indicates that while the C allele may be generally advantageous for higher-order cognitive tasks, its homozygosity could adversely affect memory consolidation or retrieval functions, which are more dependent on temporal lobe integrity.

In summary, the results demonstrate that DRD4 polymorphisms influence cognition in a genotype-dependent and task-specific manner. The C allele acts as a protective factor in several cognitive domains but may pose a risk in delayed recall when homozygous. These findings align with existing evidence of dopamine D4 receptor involvement in executive function, attention, and memory regulation, and further underscore the importance of genetic modifiers in shaping cognitive outcomes in Wilson disease.

Future research should explore the mechanistic basis of these effects, potentially involving neuroimaging and gene-expression studies, to better understand *DRD4*'s contribution to the neuropsychological heterogeneity of WD.

5.3 INDIAN GENETIC DISEASE DATABASE

- 25 monogenic diseases spread across 621 indian patients were investigated and their disease data was collected for the Indian Genetic Disease Database.
- In total, 848 mutations comprising of 247 unique mutations was updated.
- The 848 mutations contained 319 homozygotes and 300 non-homozygotes' information was updated.
- From the Populational Data, we can conclude that in the chosen 25 monogenic diseases mutation is mostly concentrated in South India (65%), then in North India (24%), followed by West India (8%) and least concentrated in East India (3%).
- Males (27%) are more susceptible to mutations than female (18%) according to the datas available. It is hard to get into a fixed conclusion as in most data the gender was unknown. (55%).
- The ratio of percentage of total no. of mutations to unique mutations was 78:22.

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My studies have two parts, as mentioned in the title of the thesis. Hence, the abstract is presented below as appropriate.

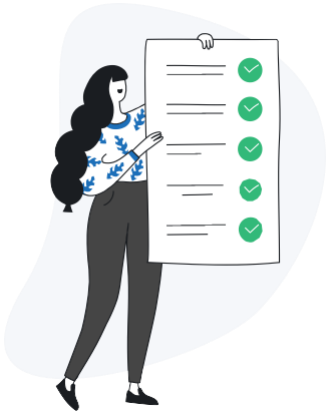
(1) Wilson Disease (WD) is a rare, autosomal recessive disorder caused by mutations in the ATP7B gene, resulting in impaired copper transport and toxic accumulation of the metal in the liver, brain, and other organs. While hepatic and neuropsychiatric manifestations are well-characterized, the cognitive aspects of WD remain underexplored. Not all patients with similar ATP7B mutations exhibit the same cognitive outcomes, suggesting a possible role of modi er genes in in uencing cognitive phenotypes. This study investigates the potential association between cognitive performance and genetic polymorphisms in two neuronal genes—GRIN2B and DRD4—in clinically diagnosed WD patients. GRIN2B encodes the NR2B subunit of the NMDA receptor, which is critical for synaptic plasticity and cognitive function. DRD4 encodes the dopamine D4 receptor, which plays a role in attention, executive function, and behavior. Both genes have been implicated in neuropsychiatric and cognitive disorders. DNA was extracted from blood samples of WD patients, followed by PCR ampli cation of selected SNP regions in GRIN2B and DRD4. Restriction digestion was carried out using AciI for GRIN2B and EcoRI for DRD4, followed by gel electrophoresis to determine genotypes. Cognitive scores were assessed using standardized neuropsychological tools. Statistical analyses were performed to evaluate the relationship between SNP genotypes and cognitive outcomes.

Preliminary ndings suggest a correlation between certain GRIN2B and DRD4 genotypes and the cognitive performance of WD patients, indicating a potential modulatory role of these variants. This study highlights the importance of considering genetic modi ers in understanding the neurocognitive variability in Wilson Disease, which may contribute to more personalized approaches in prognosis and therapeutic strategies.

(2) Indians, an endogamous population with a high incidence of recessive disorders, make up one-sixth of the world's population. However, the diseases that affect the Indian population frequently lack thorough documentation. To alleviate this challenge, the Indian Genetic Disease Database (IGDD) was released (http://www.igdd.iicb.res.in), an integrated and carefully curated repository of mutation data on common genetic diseases affecting the Indian population; the database currently contains information on 498 diseases and many individuals carrying mutant alleles of causal genes. The characterization of locus heterogeneity, the nature of mutation, clinical and biochemical information, geographic location, and common mutations are given in the published studies. The primary objective was to enhance the IGDD by integrating newly curated data and establishing the framework required for regular and systematic database updates. This involved both data deposition and infrastructure setup to support continuous improvement.

I have reviewed and compiled information from multiple publications and successfully updated the Indian Genetic Disease Database (IGDD) with curated data for 20 monogenic disorders. This process

involved extracting relevant genetic, phenotypic, and demographic data from the literature, verifying variant pathogenicity, and organizing the ndings according to the IGDD submission format



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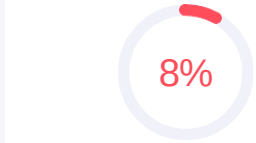

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Wilson Disease (WD) is a rare genetic disorder caused by alterations in the ATP7B gene, which disrupt normal copper metabolism and lead to its harmful buildup in the liver and other organs. It follows an autosomal recessive inheritance pattern. Clinical manifestations of WD range widely, encompassing hepatic, neurological, and psychiatric symptoms. While hepatic dysfunction often dominates the early clinical picture, neuropsychiatric features, particularly cognitive de cits, are increasingly being recognized as integral to the disease spectrum. However, the extent and nature of cognitive impairment in WD remains underexplored, with studies indicating variable involvement in domains such as memory, attention, executive function, and processing speed (Mukherjee et al., 2014). Notably, even among patients sharing similar ATP7B mutations, phenotypic variability in cognitive presentation is frequently observed, suggesting the involvement of modi er genes that may in uence disease expression. Recent literature has focused on candidate genes involved in neurotransmission pathways, particularly those regulating dopaminergic and glutamatergic signalling, which play central roles in cognitive processing.

The dopamine receptor D4 (DRD4) gene, which encodes a G-protein-coupled receptor predominantly expressed in the prefrontal cortex, has been associated with attention, decision-making, and executive function. Studies have shown that both sequence variants and epigenetic modi cations in DRD4 in uence cognitive traits in various neuropsychiatric conditions, including ADHD and bipolar disorder (Dadds et al., 2016; Zhao et al., 2015). For example, Barral et al. (2017) identi ed a promoter region variant, rs3756450, associated with enhanced perceptual speed, emphasizing the role of dopaminergic pathways in cognitive modulation.

The GRIN2B gene encodes the NR2B subunit of the NMDA receptor, a key component in glutamate-mediated excitatory neurotransmission. NMDA receptors play a vital role in regulating synaptic plasticity and are essential for cognitive functions such as learning and memory. Variants in GRIN2B have been implicated in attentional control, decision-making strategies, and phasic alertness, as shown in studies by Schulz et al. (2012) and Ness et al. (2011). These ndings underscore the gene's in uence on cognitive function in both healthy and clinical populations.

Given this context, the present study investigates the potential association between cognitive scores and polymorphisms in GRIN2B and DRD4 among Wilson disease patients. Understanding how these genes may modify cognitive outcomes could offer valuable insights into the neurogenetic mechanisms underlying phenotypic variability in WD and pave the way for more individualized management approaches.

Initially, it was challenging to access reliable data for evaluating the prevalence of speci c genetic disorders in India, as well as understanding locus and mutation-level diversity and identifying recurrent mutations, and the extent of molecular studies done in relation to disease burden. It's imperative to establish a depository of mutational data rounded by speci c clinical and other applicable information of cases from different regions of India to effectively address the situation.

Indian Genetic Disease Database (IGDD) is a forum to record case-speci c mutation diapasons of inheritable conditions in the Indian population that will help to develop individual tests and assays to detect mutations, diagnose inheritable conditions, and identify carriers. It's developed and maintained at Indian Institute of Chemical Biology (IICB), a unit of the Council of Scienti c and Industrial Research. The rst interpretation of the database was published online (Pradhan et al., 2011). Nonstop updates of present conditions and addition of new conditions are being done to make a streamlined better interpretation of IGDD. It not only contains the information of conditions but also has the information of the gene which is responsible for the complaint, along with the heredity pattern of the disease.

Plagiarized Sources

The GRIN2B gene encodes the NR2B subunit of the NMDA receptor, a key component in glutamate-mediated excitatory neurotransmission.
https://www.sciencedirect.com/science/article/abs/pii/S0920996406000764

The rst interpretation of the database was published online (Pradhan et al., 2011).
https://pmc.ncbi.nlm.nih.gov/articles/PMC7387655

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Wilson disease (WD), an autosomal recessive disorder, results from mutations in the ATP7B gene, leading to impaired copper metabolism and accumulation in vital organs such as the liver and brain. Neurological manifestations in WD are often highly variable even among patients with similar ATP7B mutations, suggesting the in uence of modi er genes on phenotypic expression.

Among potential modi er genes, GRIN2B, which encodes the NR2B subunit of the NMDA receptor, has emerged as a promising candidate due to its critical role in glutamatergic neurotransmission, learning, memory, and cognition. Schulz et al. (2012) demonstrated that GRIN2B polymorphisms, particularly rs1806201, signi cantly modulate tonic alertness without affecting other attention networks. Subjects carrying the C allele displayed faster reaction times, possibly due to more effective glutamatergic transmission and subsequent noradrenergic activation, which enhances cortical arousal and alerting mechanisms.

Similarly, Ness et al. (2011) investigated GRIN2B variants in the context of decision-making using the Iowa Gambling Task (IGT) and found a sex-dependent association of rs1806201 and rs1806191 SNPs with win-stay/lose-shift strategies. These behavioral correlates highlight GRIN2B's in uence on adaptive cognitive responses and reward-related behavior in healthy individuals. Such ndings support the hypothesis that GRIN2B polymorphisms could modulate cognitive symptoms in neurogenetic conditions like Wilson disease.

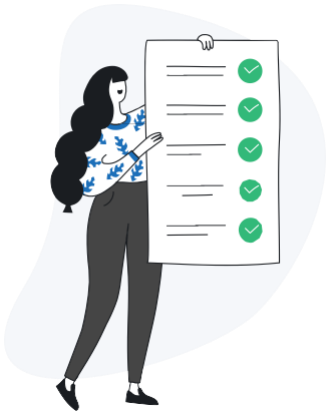
On the other hand, DRD4, a dopamine receptor gene predominantly expressed in the frontal cortex and limbic regions, plays a signi cant role in modulating executive function, working memory, and attention. In a study by Zhao et al. (2015), the rs3758653 SNP in the DRD4 promoter showed signi cant associations with cognitive performance and was also implicated in psychiatric conditions like bipolar disorder. Patients carrying this variant exhibited impaired scores in executive functioning tests such as the Wisconsin Card Sorting Test (WCST), indicating the involvement of DRD4 in cognitive control pathways.

Additionally, Barral et al. (2017) reported that DRD4 polymorphisms are signi cantly associated with slower processing speed and executive dysfunction in cognitively healthy individuals. Their meta-analysis also con rmed that the cognitive impact of DRD4 variants is domain-speci c and modulated by receptor binding ef ciency and expression regulation.

Given that both GRIN2B and DRD4 genes in uence neural circuits underpinning cognition, they are strong candidates for modulating the cognitive phenotype in Wilson disease. These genes likely act through their respective glutamatergic and dopaminergic pathways, interacting with the copper accumulation-induced neuropathology in WD.

In conclusion, previous literature substantiates the role of GRIN2B and DRD4 gene polymorphisms in regulating core cognitive domains such as attention, executive function, and decision-making. Their investigation of potential modi er genes in Wilson disease may provide new insights into genotype-phenotype correlations and cognitive outcomes in affected individuals.

Indian Genetic Disease Database is focused on the data collection, curation, and arrangement of uncommon monogenic disorders in the Indian population based on the disease of interest, the causal gene, the nature of the mutations, and the geographic location of the patients or carriers, among other factors as said by Pradhan et al. (2011). By combining vast amounts of functional and evolutionary data and creating usable tools, the related research community will get access to a valuable resource and exible platform.



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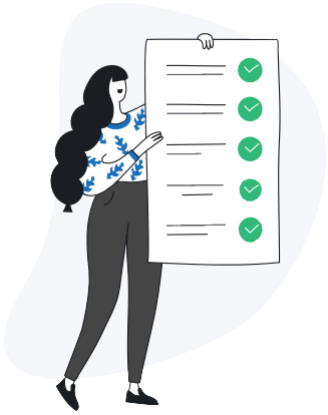
The ndings of this study indicate that polymorphisms in the GRIN2B variant are signi cantly associated with speci c cognitive traits in Indian Wilson disease patients. Among the cognitive domains analyzed, three showed statistically signi cant associations with GRIN2B genotypes ($p < 0.05$), supporting the gene's role as a potential cognitive modi er.

Notably, the T/T genotype under the recessive model was signi cantly associated with lower performance in immediate recall (Trial 1.1.1 VLT) and Con icting instruction task, identifying it as a risk genotype for these traits. These results suggest that individuals homozygous for the T allele may have impaired NMDA receptor function, which could negatively affect memory consolidation and con ict resolution, cognitive functions heavily reliant on glutamatergic signalling.

Conversely, in the Lexical Fluency (FAB) domain, the C/T and T/T genotypes combined (dominant model) were associated with better verbal uency scores, designating them as protective genotypes. This indicates a domain-speci c bene cial effect of the T allele when present in at least one copy, possibly enhancing NMDA receptor activity relevant to language and uency tasks.

Together, these results demonstrate that GRIN2B genotypes in uence cognitive performance in a domain-dependent and genotype-speci c manner. The T allele acts as a risk factor in homozygous state for memory and executive function, but shows a protective role in heterozygous form for language uency. These ndings underscore the complex role of GRIN2B in neurocognitive processing and support its consideration as a genetic modi er gene in Wilson disease.

Further investigation in larger cohorts, along with functional studies, is warranted to validate these associations and elucidate the underlying biological mechanisms.



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3.6. Indian Genetic Disease Database (IGDD): Data collection, curation, and arrangement of monogenic disorders

Here in this work, monogenic diseases, that is diseases for which a single unique gene is responsible were chosen. After choosing the monogenic diseases, we searched for research articles published with the data of Indian patients with those monogenic diseases. Data (mutation data, clinical data, etc.) were taken from those available papers.

Along with this the single gene (responsible for the disease) information was taken from OMIM (Online Mendelian Inheritance in Man), Genecard, KEGG (Kyoto Encyclopedia of Genes and Genomes), NCBI (National Center for Biotechnology Information) databases. The inheritance pattern of the diseases were also mentioned in OMIM.

The disease information, gene information and inheritance information were documented in Excel sheet, and the overall information statistics are documented in another le (Word format). Another Word le contains some broad highlights about the disease. Thus, each disease contains three les for different ways of overall presentation.

3.6.1 OBJECTIVE OF THE STUDIES DONE FOR IGDD:

• To update 25 monogenic diseases by extracting information from relevant literature from NCBI on the gene responsible for the disease along with the type of mutation, inheritance pattern, etc as well as diagnostic tests to identify mutations and carriers of the disease.

• To update gene information for 8 monogenic diseases based on OMIM (Online Mendelian Inheritance in Man), Genecards, KEGG (Kyoto Encyclopedia of Genes and Genomes) databases.

Plagiarized Sources

After choosing the monogenic diseases, we searched for research articles published with the data of Indian patients with those monogenic diseases.

https://pubmed.ncbi.nlm.nih.gov/29807575

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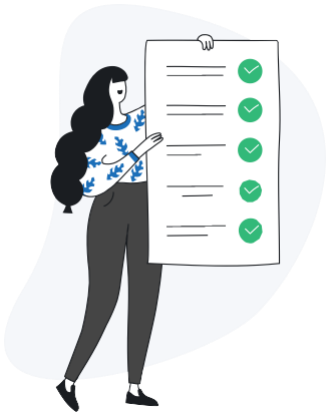
This study revealed signi cant associations between DRD4 gene polymorphisms and multiple cognitive domains in Indian Wilson disease patients, supporting the role of DRD4 as a potential cognitive modi er gene.

The dominant model of DRD4 (T/C + C/C genotypes) was found to be signi cantly associated with improved performance in verbal uency ($p = 0.041$), visuospatial ability ($p = 0.019$), and overall cognitive scores (ACE Total, $p = 0.012$). Similarly, the C/C genotype alone under the codominant model also showed a positive association with higher ACE total scores ($p = 0.026$). These ndings consistently identify the C allele—particularly when present in at least one copy—as a protective genotype, possibly enhancing dopamine receptor function in neural circuits involved in executive and spatial processing.

However, under the recessive model, the C/C genotype was associated with poorer performance in delayed cognition ($p = 0.029$), suggesting a domain-speci c risk effect when the allele is homozygous. This duality indicates that while the C allele may be generally advantageous for higher-order cognitive tasks, its homozygosity could adversely affect memory consolidation or retrieval functions, which are more dependent on temporal lobe integrity.

In summary, the results demonstrate that DRD4 polymorphisms in uence cognition in a genotype-dependent and task-speci c manner. The C allele acts as a protective factor in several cognitive domains but may pose a risk in delayed recall when homozygous. These ndings align with existing evidence of dopamine D4 receptor involvement in executive function, attention, and memory regulation, and further underscore the importance of genetic modi ers in shaping cognitive outcomes in Wilson disease.

Future research should explore the mechanistic basis of these effects, potentially involving neuroimaging and gene-expression studies, to better understand DRD4's contribution to the neuropsychological heterogeneity of WD.

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